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THE EFFECT OF CHEMICAL COMPOUNDS ON THE PRODUCTION OF THE TETANY SYNDROME

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Many workers have claimed that reactions similar to those resulting from parathyroid extirpation can be secured in certain mammals following administration by one method or another, of various chemical substances. Thus Berkeley and Beebe (2) have secured muscular twitching by intravenous injections of ammonia, which, taken in conjunction with the work of Macallum and Voegtlin (9) who observed that the urine content of ammonia increases in parathyroidectomized dogs, would seem to indicate that ammonia has some significance in the reaction of the animal to parathyroid tetany. Carlson and Jacobson (4), however, find that the increase in ammonia content of the blood of parathyroidectomized dogs falls well within the limit of experimental error. Moreover, Wilson, Stearns and Thurlow (13) have shown that the initial condition during parathyroid tetany is one of low ammonia output and it is only after the onset of tremors and convulsions that the ammonia output is increased.

Paton and Findlay (10) quote Bostock as having found a persistence of tremor after ammonia injection during anesthesia, at which time the tremors of parathyroid tetany are completely suppressed. They have also mentioned their own observations, which seem to show that the very nature of the increased tremor is primarily different. There is a fine tremor after parathyroid extirpation, a coarse, jerky tremor after ammonia injection. The question of ammonia injection as a method of producing parathyroid symptoms would, therefore, seem to be pretty well settled.

Berkeley and Beebe (2) were able to cause convulsions, snapping of jaws and muscle twitchings by the intravenous injection of xanthin. Their results could not be repeated by Voegtlin and Macallum using the same dosage and method of administration.

Biedl (3) suggested that the tetany syndrome might possibly be due to the active principle of ergot. This is not borne out by the work of Dale and Laidlaw (5) nor can any such activity be found in our own experiments.

Paton and Findlay (10) are strong advocates of guanidine or guanidine derivatives as the basis for parathyroid tetany. They point to the marked effect of a meat diet in bringing about parathyroid tetany and to Koch's (8) observation that methyl guanidine appears in the urine of parathyroidectomized animals. A full account of the history of the work of methyl guanidine and other compounds is found in their (10) work. They have shown physiologically, i.e., by tracings of tremors and the reaction of injected animals to electrical stimulation, that guanidine causes neuro-muscular phenomena similar to that following parathyroidectomy. They claim that the reactions after guanidine injection are identical with those of parathyroid tetany. In this contention they are supported by Farner and Klinger (7).

More recently Dragstedt et al (6) have shown that methyl guanidine is but one of the substances which under certain conditions may produce tetany symptoms. They have shown that parathyroidectomized animals are particularly susceptible to any kind of poisoning, and have produced what they term typical tetany seizures by stomach injections of yellow phosphorus. They have noted occurrence of tetany after bowel obstruction and have also obtained symptoms by the injection of intestinal content. In every case parathyroidectomized animals were more easily affected by such injections than were normal animals.

While it was not felt necessary to repeat the work of the investigators in this field, particularly the extensive work of Paton and Findlay and their co-workers on guanidine injections yet it seemed logical that some simple tests should be carried out in connection with our work on parathyroid tetany in the cat. To this end the experiments were planned simply as a comparison with the numerous cases of tetany which we have been able to observe. While no one who has studied the syndrome which may occur after parathyroid extirpation will lay down a single set of symptoms as typical of tetany yet the picture is fairly clear when large numbers of animals are under observation. The present writers, having become familiar with the tetany syndrome through operations upon nearly one hundred cats and dogs (11), (12) considered their experience sufficient to enable them to undertake an experiment such as presented here.

The experiments involved the use of the following substances: Methyl guanidine sulphate, methyl guanidine nitrate, trimethylamine, phosphorus, ergot, ammonia, intestinal content, creatine, creatinine and strychnine. The last named was added to the list purely for the sake of comparison with the other substances. The experiments are grouped

under three heads depending upon the method of administration: I, subcutaneous; II, intravenous injections; and III, oral administration.

I. SUBCUTANEOUS INJECTIONS: The majority of the experiments were carried out by this method, as it seemed probable that the injected materials would be absorbed more slowly and uniformly than by other methods. It appeared probable, moreover, that the end result would be the same in any case if the substance used would produce symptoms comparable with parathyroid tetany.

TABLE 1

*Methyl guanidine sulphate**

CAT	WEIGHT	AMOUNT SUBCUTANEOUSLY PER KILO	TOTAL AMOUNT	RESULTS
55	2065	0.3	0.61	Tremors, spasms and death
56	4181	0.25	1.2	Tremors, prostration, death
57	3915	0.5	1.9	No epileptiform attacks, tremors, death
58	2975	0.2	0.59	Convulsions, prostration, death
N1	3000	0.33	1.00	Epileptiform attacks, fits, tremors, opisthotonus and death

* Methyl guanidine sulphate and nitrate and trimethylamine were obtained from the chemical research division of the Eastman Kodak Co. Creatine and creatinine were supplied by Dr. W. S. McEllroy of the Department of Biochemistry, University of Pittsburgh. Cats 55, 56, 57 and 58 had previously been thyroparathyroidectomized but had failed to develop tetany probably owing to the presence of accessory parathyroids.

TABLE 2

Methyl guanidine nitrate

CAT	WEIGHT	AMOUNT SUBCUTANEOUSLY PER KILO	TOTAL AMOUNT GRAMS	RESULTS
N2	3600	0.3	1.0	Convulsions, fits, tremors, prostration and death
N3	2600	0.1	0.26	Depressed, slight tremors, dead—24 hours
N4	2760	0.15	0.414	Tremors, fits and death, 24 hours
N5	2232	0.18	0.41	Depressed, no tremors, death—2 days
N6	1642	0.2	0.33	Tremors, twitchings and death—5 hours

Normal cats were used throughout the work except in one experiment where parathyroidectomized cats were employed. The animals had been kept in the laboratory, for the major part of the time unconfined, and were thoroughly accustomed to their environmental conditions before experimentation was begun. They were fed on a scrap diet consisting chiefly of cooked meat.

The animals were injected subcutaneously in the mid-dorsal region. A few intramuscular injections were given but subcutaneous injection is to be preferred.

a. Methyl guanidine experiments. Subcutaneous injections. Methyl guanidine sulphate when given subcutaneously causes death even with a relatively low dosage. In no case was the life of the animal prolonged, as is usually the case in tetany. The onset of toxic symptoms was sudden, violent in many cases, and lacked many of the features which result when the parathyroids are removed. There has been but one case in both this and the subsequent series in which typical "paw shaking" occurred. In most cases there is a tenseness or rigidity of the body, which is lacking in the fine tremor stage of tetany. This drug acts through the efferent arc according to Paton and Findlay. This same condition occurs also after parathyroid removal but there is such a contrast in the symptomatology of tetany and of guanidine poisoning that the difference can easily be observed.

The same general results hold for this series as noted for that receiving the methyl guanidine sulphate. The typical picture of a convulsion phase, tremor with the intervening phases of tetany, is lacking after injection. It will be noted that the end result of the two series is the same, although the sequence is slightly prolonged in this second series. This is concomitant with lower dosage. In no case giving the effect of tremor or convulsion was there any indication of these effects after the longest time of the experiments above recorded.

The symptoms following injections of methyl guanidine nitrate are easily distinguished from those following parathyroid removal in cats.

Trimethylamine gave no effect on subcutaneous injection outside of a purely local reaction and a general depression of the animal. There was no effect comparable to parathyroid tetany, although the dosage in both instances was quite high. This substance, according to Koch, is found in the urine of parathyroidectomized animals in unusual amounts, as compared with normals.

This substance is known to produce liver lesions and Dragstedt, et al have stated that symptoms similar to parathyroid tetany could be produced by the administration of the substance to parathyroidectomized dogs. In our normal cases, not even a generalized condition of depression could be obtained, though we employed higher dosage than did Dragstedt. The only effect was the sloughing of the skin over the area of injection.

The results with ergot were uniformly negative. There was no depression and no tremors on subcutaneous injection. The animals remained normal throughout, except for an extensive skin slough, which followed about five days after the injection. The drug was tested physiologically and was found to show the usual reaction to smooth muscle. Massive doses

TABLE 3

Trimethylamine

CAT	WEIGHT	AMOUNT PER KILO	TOTAL GRAMS	RESULTS
N27	2100	0.3	0.6	No effect could be noted
N28	3460	0.6	2.00	Depression, sleeps continually, no tremors, no fits

TABLE 4

Phosphorus

CAT	WEIGHT	AMOUNT PER KILO	TOTAL GRAMS	RESULTS
N9	3240	0.3	1.0	No effect except for the slough of a patch of skin at the area of injection
N10	3760	0.5	2.0	No effect, as above
N11	3016	1.2	4.0	No effect
N12	2769	1.8	5.0	No effect

TABLE 5

Ergot

CAT	WEIGHT	AMOUNT PER KILO	TOTAL	RESULTS
			grams	
N13	2700	0.37	1	No effect
N14	3240	0.60	2	No effect
N15	2279	1.7	4	No effect
N16	1497	3.3	5	No effect
N17	2672	1.4	4 (oral)	No effect
N18	2460	2.4	6 (oral)	No effect

Skin slough on subcutaneous and intramuscular injections

TABLE 6

Ammonia

CAT	WEIGHT	AMOUNT PER KILO	TOTAL	RESULTS
			grams	
N19	2623	0.38	1	No effect
N20	2130	0.90	2	No effect

were used in an effort to produce some sort of a definite effect but none could be obtained. It seems highly improbable that the active principle of ergot is involved in any way in the tetany syndrome.

Subcutaneous injections of ammonium carbonate gave uniformly negative results.

Upon the basis of Dragstedt's idea that parathyroid tetany is due to absorption of toxic substances from the alimentary canal, substances probably of the nature of amines, resulting from the action of putrefactive bacteria upon proteins, these substances should be present in the intestinal contents. Dragstedt has secured tetany by injecting such contents into parathyroidectomized animals.

Although the injections, which were used in this series of normal cats are of massive dosage, no effect was obtained. The animals from which the contents were taken had been fed fresh meat for a day preceding the beginning of the experiment. The intestinal content was secured and the injections carried out as tabulated.

The series was separated into two parts. The first three animals received their injections as soon as possible after the contents were removed from the intestine. In the second, groups of two animals (N24) were injected after the material had stood for two days; (N25) were injected after the contents had been allowed to stand for five days. If decomposition products are capable of causing the effect of parathyroid tetany, the last two injections should have been potent in its production. Indol and skatol were present in large amounts.

With the exception of depression and vomiting, there was no reaction. The vomiting was not of the cachectic sort sometimes occurring after thyroparathyroidectomy.

With this substance the characteristic strychnine effect was obtained, convulsions, rigidity and death. While it may seem irrelevant to use this drug in an experiment involving a comparison with tetany, it may be noted that in several cases of methyl guanidine injection, the picture presented was quite as atypical for tetany as the sequence of events presented by strychnine poisoning. We employed it merely for comparison with the other substances injected.

II. Intravenous injections. All injections were carried out on normal cats, the jugular being opened and a cannula inserted. The materials were injected slowly in dilute solution. Two cases of methyl guanidine injection died before recovery from ether, due to too massive a dosage.

The three remaining cases of methyl guanidine injection were different in detail from each other and also were distinguishably different from parathyroid tetany. Upon recovery from the anesthetisation, one animal displayed marked tremors of the extremities. There were no tremors in the flank musculature nor were there convulsive movements of any sort. The animal was prostrate for six hours after injection after which recovery was normal. In the other two cases, the symptoms ran about the same course of events. Prostration was of longer duration in one animal in which a marked depression was noted. No tremors or convulsive move-

TABLE 7

Intestinal contents

CAT	WEIGHT	TOTAL AMOUNT	RESULT
		cc.	
N21	2941	5	No effect
N22	3617	10	Depressed, vomited, no tremors
N23	1920	5	Depressed, sick, no tremors, recovered
N24	1614	5	Very sick, vomited violently, depressed, recovered
N25	3290	5	Very sick, vomited, depressed, recovered

Injected with colon residue in which putrefaction was complete

TABLE 8

Creatine

CAT	WEIGHT	AMOUNT PER KILO	TOTAL	RESULTS
			grams	
29	2732	0.36	1	No effect
30	3340	0.90	3	No effect

TABLE 9

Creatinine

CAT	WEIGHT	AMOUNT PER KILO	TOTAL	RESULTS
			grams	
31	3760	0.40	1.5	No effect
32	3120	0.93	3.0	No effect
33	1691	1.7	3.0	No effect

TABLE 10

Strychnine

CAT	WEIGHT	AMOUNT PER KILO	TOTAL	RESULTS
			cc.	
60	1150	9.9	10	Dead five minutes
N7	1956	2.6	5	Strychnine convulsions—death
N8	2642	1.1	3	Convulsions—death

Death characterized by rigidity, which has a sudden onset after a few tremors

ments were noted in this animal in a period of recovery which lasted eight days. The remaining animal showed a marked depression with complete absence of tremor or convulsive attack.

Ergot was inert in all injections and no effect could be elicited.

Trimethylamine and ammonia both give a characteristic reaction, which

is different from parathyroid tetany. Both substances bring about coarse, jerky tremors of the extremities and body musculature while the animal is under anesthesia. This reaction occurred in both light and deep anesthesia. An overdosage or too rapid administration of either of these drugs will bring about death. The coarse jerky tremors persisted for some time after cessation of respiration and heart beat and give way to a ridigity similar to that obtained after strychnine. The tremors of parathyroid tetany disappear under deep anesthesia.

TABLE 11

Intravenous injections

SUBSTANCE	NUMBER OF CATS	DOSAGE PER KILO GRAMS	RESULTS
			Death in high dosage No tetany symptoms
Methyl guanidine sulphate	3	0.4 to 0.1	Depression in lower dosages
Methyl guanidine nitrate	2	0.6 and 0.1	No tremors or induced tetany
Trimethylamine	4	0.1 and 0.02	Death in high dosage characterized by rigidity
Ammonia	2	0.6 and 0.2	Coarse, violent, jerky tremors persisting under anesthesia
Ergot	3	2.0 and 0.5	No effect

TABLE 12

Oral administration

SUBSTANCE	NUMBER OF CATS	DOSAGE PER KILO GRAMS	RESULTS
Methyl guanidine sulphate	2	2.0 and 1.0	Depression without tremor or convulsion. Uniform recovery
*Methyl guanidine nitrate	2	0.8 and 0.5	
Trimethylamine	4	0.8 and 0.02	Nausea and depression, no tremors, no convulsions
Ammonia	3	0.7 and 0.01	
Ergot	6	4.0 and 0.7	No effect
Phosphorus	3	3.0 and 0.5	No effect

* Eight additional animals were given doses up to 2 grams per kilo without fatal results.

III. ORAL ADMINISTRATION. Administration of the drugs was accomplished by means of a small stomach tube introduced under anesthesia. This method was resorted to when the impossibility of administration by other methods had been demonstrated. The animals were closely observed for long periods after administration to be sure that none of the injected materials were lost by regurgitation.

The methyl guanidine series gave negative results in all of our cases. Dosage which would cause almost immediate reaction by subcutaneous injection induced merely a depression from which the animal recovered comparatively quickly. Trimethylamine and ammonium hydrate give a reaction of decided depression and nausea but no tremors or convulsive attacks.

Ergot and phosphorus were negative although given in massive dosage (cf. table).

Discussion. Our attempts to produce symptoms comparable to those of parathyroid tetany by means of injection or the administration of various substances to normal animals have failed in every case. Methyl guanidine sulphate and nitrate injected subcutaneously cause symptoms which tend to simulate those induced by parathyroid removal but the symptomatology and sequence present somewhat different pictures. There are individual effects produced by the guanidine series, which strongly simulates the response following parathyroid extirpation in the cat but the aggregate of symptoms in the two cases is hardly comparable.

It is to be emphasized that our work was done upon normal animals. It is possible that animals which have undergone a parathyroidectomy and which exhibit signs of parathyroid insufficiency may be acted upon more readily by these, and other substances. The evidence seems to point, in nearly all biological groups, to a lowering of resistance after parathyroidectomy not only to specific poisons but also to infection.

The inert condition of ergot and phosphorus in all series of experiments is constant. There was no effect of any sort observed which in any way could be compared with the symptoms of parathyroid tetany.

Trimethylamine and ammonia give perfectly definite results. Their effects are easily differentiated from those of the other substance and also from the effects after parathyroid extirpation. The coarse jerky tremors of the extremities, the tenseness and rigidity with lack of tremor in the body musculature together with the continuance of these reactions in deep anesthesia and even after cessation of heart beat and respiration are so different from parathyroid tetany that there is no possibility of confusion.

The method of administration plays an important part in the sequelae obtained. Methyl guanidine has practically no effect when administered by stomach tube; it is in the subcutaneous series that its action most nearly simulates tetany.

Trimethylamine and ammonia are most effective when administered intravenously. Their action, however, is not shown by other methods of administration.

Creatine and creatinine although related to the guanidine series failed to give any reaction with subcutaneous injection. Unfortunately, the amount of these substances available at the time of experiment was limited so that their effect in other series could not be noted.

Intestinal contents are non effective in subcutaneous injection. Stomach tube administrations were unsuccessful as the majority of the material was lost during recovery from anesthesia. The retained portion was sufficient to give a marked depression but there was no other effect noted.

While the reactions of cats to methyl guanidine injections and the reactions obtained after parathyroidectomy may have some effects in common, the results are essentially different in so many phases, that methyl guanidine intoxication can hardly be regarded as a primary causative factor in the production of parathyroid tetany.

SUMMARY

The reactions of normal cats to subcutaneous and intravenous injections and oral administration of certain substances, viz., methyl guanidine sulphate, methyl guanidine nitrate, trimethylamine, ergot, phosphorus, intestinal content, ammonia, creatine, creatinine and strychnine have been observed.

The symptoms occurring after injection of these substances were hardly comparable to the reactions of an animal suffering from parathyroid tetany.

The substances vary in effect according to their method of administration, methyl guanidine showing most marked reactions after subcutaneous injection and practically no effect after oral administration. Trimethylamine was active only when injected intravenously.

Ergot, phosphorus, creatine and creatinine were uniformly inert in all series.

Four thyroparathyroidectomized cats which had failed to show tetany symptoms due probably to the presence of accessory parathyroids, did not show tetany symptoms when injected subcutaneously with methyl guanidine sulphate.

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OXYGEN CONSUMPTION DURING REPEATED SLIGHT HEMORRHAGES

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The work presented here is the effect of successive small hemorrhages on the total oxygen absorption of an animal in unit time. A résumé of the literature shows that nearly all previous experimentation involved considerable losses of blood (1). We therefore started with small bleedings and bled successively or continuously until 3 to 4 per cent of the body weight had been removed. This procedure was done within a relatively short period of time, quite comparable to the length of time that patients are kept on the operating table or before adequate aid is given in injuries involving hemorrhage. It must be emphasized here that this study is one during the hemorrhagic stage only, and therefore a study of acute change. The equally important post-hemorrhagic phenomena are not considered in this paper.

Many years ago Bauer (2) studied oxygen consumption from day to day in dogs after bleeding at once large amounts, 2 or more per cent, of the body weight. On the day following the bleeding there was a decrease in oxygen usage as determined by the Voit respiratory chamber method. Students of basal metabolism could point out the errors in this method. Later Finkler (3) noted that there was no immediate influence on an animal following bleeding. Fredericq (4) obtained no constant or large variation. The fasting rabbit occasionally showed an immediate temporary decrease. Guerber (5) showed that there was a change only after large hemorrhages. More recently Murlin and Greer (6) using the Benedict respiration apparatus and taking blood samples in three tracheotomized dogs under chloretone narcosis found slight changes after bleeding 1 to 2 per cent of the body weight. In 1920 Aub (7) reported the effect on metabolism of hemorrhage induced in fully anesthetized and tracheotomized cats. A low blood pressure, following hemorrhage alone (never more than 1 per cent of the body weight) may be associated with only a slight drop in metabolism, temporarily lower it, or have no immediate effect on the metabolic rate. Doi (8) noted a drop in oxygen consumption in urethanized animals that were bled from the femoral artery. There was also an acceleration of the respiration and pulse.

METHODS. Dogs were used in all experiments. After the operative procedure was performed under ether the tracheotomized animal was connected to an oxygen container, equipped with a carbon dioxide absorber. The oxygen container recorded the rate and depth of respirations as well as the amount absorbed by gradual exhaustion of the container. The container was always calibrated at each experiment. The respiratory rate was greater than the normal at the start of most experiments. Macleod has noted that the slight resistance to the movement of the air probably causes a greater degree of alteration in intra-alveolar pressure and

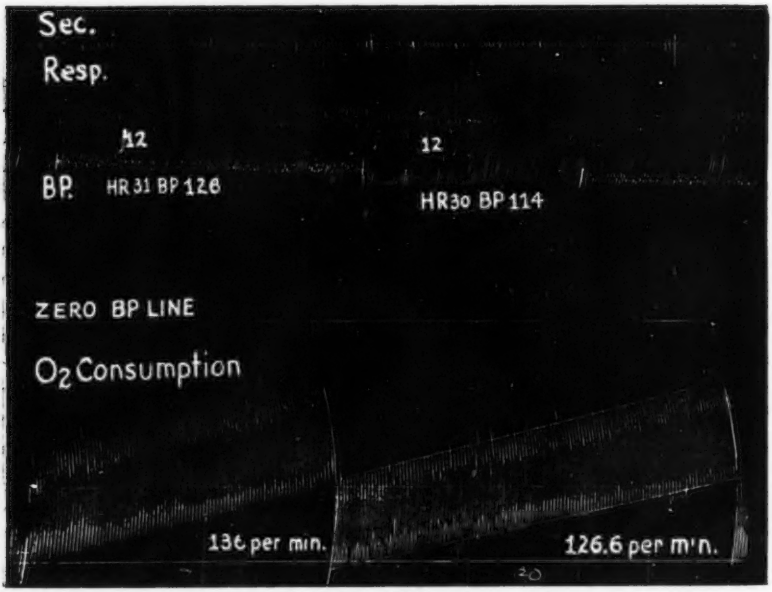

Fig. 1

consequently a stimulation of afferent respiratory fibers. One must also consider the pressure of excess CO_2. In addition tracings were taken of the carotid blood pressure and of the respiratory changes by a bladder placed about the chest and connected by air transmission to a recording tambour.

The oxygen container used is that described by Jackson (9). The readings given by the tracing method were checked from time to time by a stop-watch. Measured amounts of oxygen were supplied. The first tracings were taken within fifty to sixty minutes after the beginning of anesthesia. In figure 1 an example of part of an experiment is shown.

The experiments fall into two series, *a*, animals etherized by leading ether from a burette into the oxygen container to be inhaled with the oxygen, and *b*, animals anesthetized by 9 per cent paraldehyde injected intravenously. These in turn are respectively divided into control groups and bleeding groups.

The bleeding from the femoral artery was done repeatedly at short intervals in amounts of $\frac{1}{8}$ to $\frac{1}{3}$, mainly $\frac{1}{4}$ of 1 per cent of the body weight. Later in the individual experiments unit bleedings of 1 per cent of the body weight were common. In the dog the average total blood volume is approximately 10 per cent of the body weight (10), therefore a bleeding of 1 per cent of the body weight equals 10 per cent of the total blood volume.

TABLE 1

Control experiments

Average mean variation in per cent of normal

EXPERIMENT	PROCEDURE	BLOOD PRESSURE	HEART RATE	RESPIRATORY RATE	OXYGEN ABSORPTION PER MINUTE SCALE
OC IX	Ether	3.56	7.29	9.25	8.70
X	Ether	2.87	5.0	7.75	10.00
XII	Ether	2.00	*	1.70	10.00
XIV	Ether	2.10	3.19	7.40	6.03
OC XV	Paraldehyde	2.45	2.0	5.90	9.60
XVI	Paraldehyde	3.58	5.27	7.66	3.16
XIX	Paraldehyde	4.8	4.20	8.35	3.96
Maximum	Variat. ±	4.8	7.29	9.25	10.00
Minimum	Variat. ±	2.0	2.0	1.70	3.16

*Could not be calculated.

DATA. *Controls.* The control experiments fall into two groups: *A*, ether, four experiments; and *B*, paraldehyde, three experiments. No bleeding was done in these at all. Their duration was from 110 to 225 minutes. There was an additional ether experiment, no. XI, in which no bleeding was done, until after an interval of 160 minutes. Adding this to the others really gives us five ether control experiments.

Curves are given in figure 2, showing the variations in blood pressure, heart rate, respiratory rate and oxygen consumption in ether control experiment XIV which lasted nearly four hours. The changes in any of the mechanisms observed show an average mean variation that is not in excess of 7.40 per cent. Quite parallel in results to this ether control experiment is the paraldehyde control no.XIX. In no. XIX, the average mean variations do not exceed 8.35 per cent in any mechanism. Table 1 summarizes briefly the average mean variations in per cent of the normal. The maximum for the blood pressure was 4.8, for the heart rate it was 7.29,

TABLE 2

NUMBER		PROCEDURE	NUMBER OF EXPERIMENT	WEIGHT IN KILOGRAM	NORMAL					DURATION OF EXPERIMENT IN MINUTES AND PER CENT OF NORMAL OXYGEN CONSUMPTION PER MINUTE (O_2 CONSUMPTION PER MINUTE = 100 PER CENT)																AVERAGE PERCENTAGE DROP IN O_2 CONSUMPTION AFTER BLEEDING			TOTAL AMOUNT OF BLOOD BLED IN PER CENT OF BODY WEIGHT
					Oxygen consumption per minute in cubic centimeters	O_2 consumption per kilogram per minute	Surface area in square meter	O_2 consumption per square meter in cubic centimeters per minute	Duration of experiment in minutes	0 minute	50 minutes	60 minutes	70 minutes	80 minutes	90 minutes	100 minutes	110 minutes	120 minutes	130 minutes	140 minutes	150 minutes	160 minutes	170 minutes	240 minutes	360 minutes	1 per cent	2 per cent	3 per cent	
1			IX	14.8	131	8.86	0.67	213	60	100		110.7	109	105.8	92.3	100.4	81.6											
2			X	11.5	117.9	10.2	0.57	206	165	100	97.0	106			93.8	117		95.1										
3		Ether Controls	XII	13.2	135	10.2	0.62	215	210	100			102	89 73	98 78		86 100 100	98	103 103 96			94	116 120						
4			XIV	5.6	85.3	15.2	0.35	241	225	100		94.9	97				100.8	104.1		95	102.6	109.8	93.7 109.7						
5	A		II	7.6	97.3	12.8	0.43	224	120	100	*0.33 69.6	0.33 68.4	0.33 86.9	0.33 65.9	1.0	67.8	1.0								13.1	31.1	32.2	3.0
6			III	12.2	129.4	10.6	0.59	217	135	100	*0.125–0.125 100–97	0.25 100	0.25–0.25 79–86	0.5 79	0.5 78	1.0 88		1.0 74							14.0	22.0	12.0	4.0
7			IV	7.8	84.9	10.8	0.44	192	180	100			0.25 83.9	0.25 78.2		0.25 78.2	0.25 72.9	1.0 61		1.0 53.6					27.1	39.0	46.4	3.0

8		Ether and Bleeding	V	6.6	58.6	8.83	0.39	187	120	100	*0.25 83.6	0.25 110	0.25 90.6	0.25 87.7	1.0 79.7	1.0 88.4												12.3	20.3	11.6	3.0
9			VI	5.5	74.6	13.5	0.34	213	90	100	*1.0 56	1.0 83.6	1.0 27.3														44.0	16.4	73.7	3.0
10			VII	8.1	99.6	12.29	0.45	220	180	100			0.25 109.9	0.25 97.6	0.25 91.5	0.25 74.6												25.4			1.0
11			VIII	10.6	106.6	10.0	0.54	197	120	100	*0.25 89.1	0.25 93.8	0.25 87.7	0.25 75	0.25-1.0 84-56												25.0	43.7		2.25
12			XI	9.4	119.4	12.7	0.49	239	360	100					100						100			1.0 82.6	1.0 50.9	17.4	49.1		2.0
13			XV	5.9	130.8	21.2	0.34	379	165	100				19.7 77.2		94.8 109.3	105.5	97.8	102		121								
14		Paraldehyde Controls	XVI	5.7	89.5	15.7	0.35	250	180	100	91.2 102.4		109		96.6		101.6		152	100.5		97.7	100.1						
15			XIX	7.9	124.9	15.8	0.44	281	150	100			99.9	98.1	99.9		98.1 90.4	103 104 111	109 101 99	101 101									
16		Paraldehyde and Bleeding	XVII	5.0	105	21.0	0.32	317	180	100					*0.33 95.2		0.33 94.2	0.33 95.2	0.5 95.2		1.0 79	1.0 76.6				4.8	21.0	32.4	3.5
17			XVIII	4.4	119	27.0	0.30	395	135	100	*0.25 111.7	0.25 105.8	0.25 110.9	0.25 95		1.0 86.4	1.0 86.4		1.0 53.9						5.1	13.6	13.6	4.0

*Italicized figures = part of 1 per cent of body weight bled.

for the respiratory rate it was 9.25, and for oxygen absorption it was 10.0. In none of these mechanisms is the maximum variation a marked one.

As the oxygen absorption of these animals is of particular interest in this problem an analysis of the normals in all the experiments as shown in table 2 is of value. An index to the accuracy of the method can be ob-observed when comparisons are made with oxygen consumption data obtained by other technique and investigations. In the ether experiments the dogs weighed from 5.5 to 14.8 kgm. The surface area of these animals, using Meeh's formula, ranged from 0.34 to 0.67 square meter. The oxygen consumption per kilogram per minute was 8.83 to 15.2 cc., and per square meter per minute it was 187 to 241 cc. On comparing these last figures with those prevailing in the paraldehyde series it can be seen that the oxygen consumption is considerably higher in the latter. For example, the oxygen consumption per kilogram per minute was 15.7 to 27.0 cc., and per square meter per minute it was 250 to 395 cc. This probably shows that in the ether series movement and tonus was cut down to a minimum.

To assure ourselves that the normal oxygen consumption data in the ether anesthesia experiments particularly are fairly basic, and that extraneous factors as muscular movement was practically eliminated; in other words, that a basal metabolic state had been approached, data are cited which are taken from other investigators. These may serve as a basis of comparison.

Our figures for the ether series range from 8.83 to 15.2 cc. per kgm. per minute or 187 to 241 cc. per square meter per minute. Magnus-Levy (11) in his classical monograph on the specific dynamic action of the foodstuffs reports that his tracheotomized dog, weighing 27.5 kgm., had an oxygen consumption of 5.73 cc. per kgm. per minute or 141 cc. per square meter per minute. It will be remembered that we also tracheotomized our dogs. Further comparative data are indicated in table 3. They range from 5.05 to 19.1 cc. (weights, 4.7–28 kgm.) as compared with our 8.83 to 15.2 cc. (weights, 5.5–14.8 kgm.).

The paraldehyde series have already been referred to as having rather high oxygen consumption figures. These therefore do not represent satisfactory absolute or basal data. They are sufficient for relative purposes for the same high figures prevail in both the controls and bleeding experiments, and they can therefore be compared with each other.

Effects of hemorrhage. Before taking up each group separately the criteria of the effects of bleeding will be indicated. It has been shown by Meek and Eyster (1) that in the intact dog hemorrhage amounting to an average of 2.0 per cent of the body weight (equivalent to 20 per cent of the blood volume) is necessary before a crisis occurs in the circulatory mecha-

nism. This breaking point is manifested by a reduced diastolic size or cardiac filling, a constriction of cutaneous venules and capillaries, usually a significant increase in heart rate, a fall in arterial blood pressure, and a faster respiratory rate. This does not necessarily mean that all of the three factors were altered simultaneously in every experiment. In our experiments we followed only three of these factors—arterial blood pres-

TABLE 3

AUTHOR	CITATION	METHOD	WEIGHT OF ANIMAL	SURFACE IN	O_2 CONSUMED PER MINUTE	
					Per kilogram	Per square meter
				sq. m.	*cc.*	*cc.*
Our data*			5.5–14.8	0.34–0.67	8.83–15.2	187–241
Magnus-Levy	Pflüger	Gas anal.	27.5		5.73	141
Murlin and Greer	Am.J.Physiol.	Gas anal.	10.2		6.57	127
Hill (12)	Recent Advances		6.2		15.2	313
Richet (13)			4.7 –28.0		1.6–19.1	
Williams, Riche and Lusk (14).		Same dog	13.78–16 07		6.7–12.2	
Lusk and Riche (15)			12.07		7.48	
Atkinson and Lusk (16)		Basal metab.	11.77		5.05	
Barcroft (17)	Erg.d.physiol. 1908, vol, 7, 699.		7.5		5.33	

*Our readings are a little high throughout because of a constant over-estimate made by calculating apparent total oxygen delivery from the graduate cylinder and not correcting for the space occupied by the glass tube.

sure, pulse rate and respiratory rate. In addition the oxygen consumption per minute was determined, and the respiratory depth observed. The responses to bleeding of individual animals permit us to divide them into groups, as in all studies that have been made on the effects of anoxemia, no matter how produced, there being no single, typical reaction for all of the physiological mechanisms involved. Barcroft (18) has designated our type of anoxemia as anemic anoxemia. The question of anemic anoxemia will be taken up in greater detail in the discussion.

Ether anesthesia and bleeding. In three experiments, group A, figure 3, (II, VIII, XI) no marked change in the pulse rate occurred. However, the blood pressure and oxygen consumption dropped in all three, and the

respiratory rate was decreased in two and did not change in one. In three other experiments, group B, figure 3, a fast pulse occurred with concomitant drops in the respiratory rate, blood pressure and oxygen consumption. In experiment IV, group C, figure 3, both a fast heart and respiratory rate prevailed while the blood pressure and oxygen consumption were decreased. In experiment VII a rapid pulse occurred with a slight fall in blood pressure, and an appreciable drop in both the respiratory rate and oxygen consumption. Therefore in this set of ether bleeding experiments a marked drop in oxygen consumption occurs in every single one, with a fall in blood pressure, and this drop finally ranges from approximately 85 to 35 per cent of normal.

Figure 3 illustrates these changes graphically, according to the groups designated above.

The drop in oxygen consumption is progressively more and more as bleeding advances from 1 to 3 per cent of the body weight. With 1 per cent bleeding the oxygen consumption ranges from 56 to 87 per cent of the normal. The pronounced decreases occur with rapid bleeding of 1 per cent, or repeated bleeding of smaller amounts within a relatively short time interval. With 2 per cent bleeding the oxygen consumption is 51.9 to 79.7 per cent of the normal. In one case (exper. VI) the oxygen absorption recovered and rose from 56.0 to 83.6 per cent of normal. In five experiments with a bleeding amounting to 3 per cent of the body weight the oxygen consumption ranged from 26.3 to 88.4 per cent of the normal. The decrease in respiratory rate which occurred in seven of the eight experiments was accompanied by increases in respiratory depth, showing that there was no exhaustion of the respiratory centers. In some experiments the increase was followed by a decrease, probably due to paralysis of the respiratory center from oxygen want. In experiment IV when the respiratory rate was markedly increased there was a thirty per cent decrease in depth of respiration.

Paraldehyde anesthesia and bleeding. In addition to the three controls, two hemorrhage experiments, XVII and XVIII, were performed. These two are shown diagrammatically in figure 4.

Examination of the sketches reveals the fact that these two experiments fall into groups A and B, which also form the two chief groups for the ether and hemorrhage experiments.

To prove that our method of administering ether did not interfere with the oxygen consumption calculations, we gave a solution of paraldehyde in normal saline intravenously and found that here also hemorrhage of 2 per cent of the body weight produced an appreciable drop in the oxygen consumption.

Discussion. As here shown, there seems to be no *marked* decrease in oxygen consumption until bleedings of about 1.5 to 2.5 per cent of the body

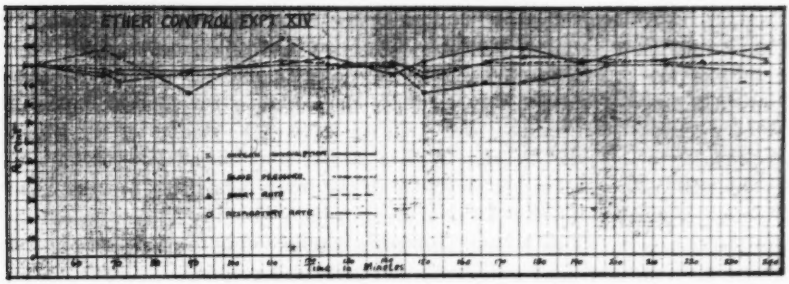

Fig. 2

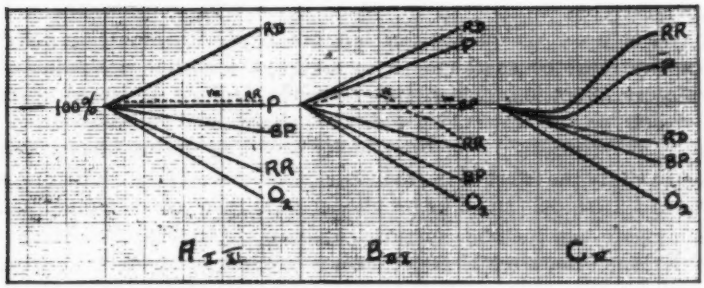

Fig. 3. P = pulse rate; BP = blood pressure; RR = respiratory rate; RD = respiratory depth; O_2 = oxygen consumption.

The broken lines indicate a variation from the group for that particular mechanism.

The roman numeral indicates the experiment.

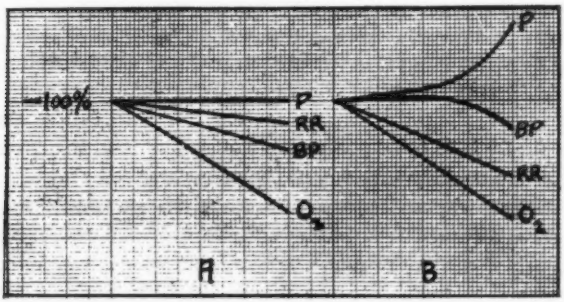

Fig. 4. P = pulse rate; BP = blood pressure; RR = respiratory rate; RD = respiratory depth; O_2 = oxygen consumption.

The broken lines indicate a variation from the group for that particular mechanism.

The roman numeral indicates the experiment.

weight have occurred. Meek and Eyster have also indicated that not until about 2 per cent of the blood has been taken out does a circulatory and respiratory crisis occur. Up to this point an efficient protective mechanism is present and possibly, as they suggest, it is a decreased capacity of the venous cisterns together with an increased velocity of the blood returning to the right heart. With a 20 per cent loss of the total blood volume involving a marked decrease in the oxygen-carrying power of the blood it is conceivable that there is an anoxemia added to the other factors in the situation. This anemic anoxemia is reflected in the reduced oxygen consumption, increased pulse rate and increased respiratory depth. The last two are compensatory factors.

It is true that the results cannot be viewed strictly as a reduction in the oxygen-carrying capacity of the blood alone. In addition, the radical removal of a certain quantity of nutrient *fluid* with markedly colloidal properties must be considered as a factor of importance conducive to circulatory alterations, which may be mechanical, as decreased filling of the vessels, or chemical. This additional phase could be taken care of by injecting centrifuged serum in amounts equalling the blood removed in order to study the effect of the reduction of functional hemoglobin material alone as did Whipple and his co-workers (10) in their blood volume studies. Furthermore, our method comprises a study of the acute condition, and not the condition after bleeding until a *readjustment to the normal* takes place.

The reduced oxygen absorption may be due to two factors. One of these is the reduced oxygen-carrying capacity of the blood typified by the reduction in red blood cells, and the other is an actually reduced oxygen demand of the tissues as a result of altered physico-chemical conditions after a primary stimulation. The rapid pulse which prevails in the bleeding experiments increasing the coefficient of oxygen utilization is a factor which helps cut down the oxygen reserve. This is quite an appreciable component as Murlin and Greer demonstrated and as Benedict and Talbot (19) showed in their study of oxygen usage in infants. In three of our ether experiments the pulse was not increased in rate, but oxygen absorption was reduced to 51 to 66 per cent of the normal, and to a greater extent than in the five experiments where the pulse was very rapid. The fast heart, therefore, seems to increase the rate of oxygen usage for some time, as would be expected.

These types of cardiac response resemble in essential detail two of the groups described by Schneider and Truesdell (20) in their anoxemia work, those with an excellent respiratory reaction and those in which a circulatory compensation is lacking.

In our experiments the physiological adaptations consist apparently in an altered rate of blood flow and increased ventilation of the lungs.

These occur simultaneously with the beginning drop in oxygen consumption. In other words, a crisis occurs. There is no warning here by the occurrence of certain compensatory reactions to low oxygen as noted by Gregg, Lutz and Schneider (21) and, of course, the parallelism with purely low oxygen experiments breaks down by the fact that there can be no appreciable increase in the hemoglobin in bleeding experiments within the time under study. If any increase occurs within ten to thirty minutes it is quite exceptional. Evidently an increased general blood flow must occur because of the decreased total blood volume during the compensatory stage in hemorrhage and later on the break in compensation is probably accompanied by a reduced general volume-flow of blood. What happens in an isolated organ like the submaxillary gland (22) is merely suggestive.

In the anesthetized animal the body temperature drops gradually producing an increased tonus of the skeletal musculature. Guthrie (23) has shown that under these conditions a drop in temperature increases metabolism. These two factors probably balance each other.

Although blood-letting or anemia is the easiest method of producing a reduction in the oxygen supply of the body, still, as we have already noted, it goes beyond the production of anoxemia only in that it also depletes the vascular system of circulatory fluid, irrespective of its oxygen-carrying capacity. Therefore, the four usual ways of physiological adaptation or acclimatization to anoxemia consisting of *1*, increased respiration, *2*, increased percentage of hemoglobin, *3*, increased rate of blood flow, and *4*, increased oxygen tension in the blood, do not strictly apply here.

In hemorrhage for a period of time more blood must flow to the tissues for the blood contains less oxygen per unit volume. This increased flow brings the required amount to maintain basal metabolism.

In uncomplicated anoxemia a good response is evidenced by an increased respiratory volume per minute, a fast pulse, a rise in both the systolic and pulse pressures, and a moderate fall in diastolic pressure. In hemorrhage all of these may occur, as evidence of an excellent reaction, but an important requisite—increased hemoglobin—to maintain compensation is lacking.

Schneider (24) calls attention to the fact that a crisis occurs in nearly all subjects in altitude experiments, when the altitude is about 19,000 feet above the sea level, which is equivalent to an atmosphere containing 10.16 per cent oxygen, and that a large number of individuals show appreciable effects at 14,000 feet or 12.28 per cent oxygen. The changes in both the altitude investigations and in rebreathing experiments were at the rate of 1000 feet a minute or 0.40 to 0.85 per cent of oxygen reduction in the atmosphere for every thousand feet. Studies of the oxygen unsaturation of these levels would be instructive.

In hemorrhage a crisis or active compensatory processes occur when approximately 20 per cent of the total blood volume is removed. It may eventually be shown that the oxygen supply to the tissues in both these conditions is substantially the same.

SUMMARY

The oxygen absorption was determined in dogs under ether or paraldehyde anesthesia before, during and after 0.12 to 1.0 per cent of the body weight in blood was removed at intervals so that 10 to 40 per cent of the total blood volume in an average of 2 to 3 hours was taken.

There is no *marked* decrease in oxygen consumption until bleedings of about 1.5 to 2.5 per cent of the body weight or 15 to 25 per cent of the blood have been done. It is at this stage that other investigators have demonstrated the occurrence of a circulo-respiratory crisis. Our curves in figures 3 and 4 for oxygen consumption merely indicate the drop in the experiments so that comparisons could be made of the other functional reactions.

It is a pleasure to thank Doctors Eyster, Meek and Schneider for their criticism in this problem.

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STUDIES ON THE PATHOGENESIS OF TETANY

IV. The Tetany of Oestrus, Pregnancy and Lactation

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It was early discovered through the observations of Zanfrognini, Erdheim, Thaler, Adler, Vassale, Massaglia, Halsted and others, that pregnancy may induce severe tetany in dogs that have survived a partial parathyroidectomy for long periods without such symptoms. It has also been definitely established that pregnant bitches develop severe tetany and die much sooner following complete thyro-parathyroid extirpation than do males or non-pregnant female dogs. The experiments of Carlson (1) and Werelius (2) are particularly instrucive. The great majority of their pregnant dogs died in severe tetany within thirty-six hours following the operation. Non-pregnant dogs, if fed on mixed diets of meat and vegetables, usually survive four to six days and sometimes much longer.

There are a number of reports in the clinical literature (3) where an apparently spontaneous tetany has repeatedly recurred during pregnancy and lactation, although entirely absent at other times. Thomas (4) cites a case in which tetany appeared in six successive pregnancies. It always occurred during the latter part of pregnancy. The early part of pregnancy seemed to have a beneficial effect. Exposure to cold was thought to be an exciting factor, the attacks coming on chiefly in winter. Menstruation is also cited as an exciting factor which may transform a latent into an active tetany. It is not uncommon to observe slight symptoms of tetany (hyperpnea, salivation and muscular twitchings) in apparently normal bitches during the period of heat.

The sudden onset and uniform severity of tetany in parathyroidectomized pregnant animals and the recurrence of tetany in partially parathyroidectomized animals during oestrus, pregnancy and lactation, are interesting and significant facts. It is quite evident that the fetal parathyroids cannot compensate for the absence of the mother's glands although, as Carlson (1) remarks, the parathyroids must become functional in the fetus a considerable time before birth, because there is no record of parathyroid tetany in prematurely-born infants. Spasmophilia is

however more common in these children than in those born at full term. Either the parathyroids do not function by means of an internal secretion or the hormone cannot pass through the placenta. In the latter event the hormone must be quite different from the hormone of the pancreatic islets, insulin, which Carlson (5) has shown passes from the fetal to the maternal blood stream. It also appears evident that the tetany poisons in the maternal blood do not pass into the fetal circulation and become destroyed or neutralized at least to any appreciable extent.

To those investigators who postulate an etiological relationship between a decreased calcium concentration of the blood and tetany, it would appear obvious that such conditions as pregnancy and lactation, in which there is a drain on the maternal calcium, would induce or aggravate tetany. The tetany which recurs during oestrus or menstruation in primates would then appear to be a separate entity.

In the previous reports (6) of this study on the pathogenesis of tetany, a description has been given of a method which will prevent the onset of tetany or depression in dogs following complete removal of the parathyroid glands and will permit these animals to survive indefinitely and in comparatively good condition. The principle of the method involves the prevention of bacterial proteolysis in the intestines by special diets. After intestinal putrefaction has been prevented for periods of three to six weeks and the parathyroidectomized animal kept free from tetany during this time some readjustment takes place so that the animal may thereafter remain in good condition and free from tetany on ordinary mixed laboratory diets. The resistance of these parathyroidectomized dogs to a considerable variety of poisons is markedly reduced. They may be profoundly intoxicated by amounts of toxic chemicals which will not affect normal animals. A number of factors, such as the feeding of large amounts of meat, the occurrence of constipation, exposure to high temperatures, excessive muscular exercise, the occurrence of oestrus, sexual excitement and infections, may induce tetany or depression in these animals although rarely causing disturbance in normal dogs. These facts suggest that whereas the chief source of the tetany poisons in the blood of the parathyroidectomized animal has been the gastro-intestinal tract, under certain special conditions they may come from other places. This is particularly indicated in the following experiments, where tetany recurred in completely parathyroidectomized animals during pregnancy.

Dog 38. Adult, female dog. Weight 10 kgm.

July 20, 1922: Diet, white bread and milk ad libitum, lactose 60 grams per day.

August 2: Feces liquid, odorless, acid to litmus. Complete thyro-parathyroidectomy.

August 3–4: Condition good. No tetany.

August 5: Severe tetany (tonic and clonic convulsions); 500 cc. of 20 per cent lactose solution were given by stomach tube. In a short time there was a profuse watery bowel movement. Tetany subsided.

August 6–7: Condition good. No tetany.

August 8: Occasional jerking movements of the forelegs.

August 17: Slight tetany (hyperpnea, salivation, spasticity, tremors in the temporal and neck muscles). Very warm day. Five hundred cubic centimeters of Ringer's solution given intravenously. Recovery.

August 18–October 30: Condition good. No tetany.

October 31–November 9: Dog is in heat. Has been repeatedly observed in copulation. During this period there has developed a marked enopthalmos and blepharospasm but no convulsions.

November 10: During copulation with male there was marked excitement and spasticity but no convulsions. Immediately after copulation a second male was brought in. During copulation with the second male a violent convulsion occurred (tonic and clonic generalized convulsions, salivation and hyperpnea). Five hundred cubic centimeters of Ringer's solution given intravenously; 500 cc. of 20 per cent lactose solution given by stomach tube. Recovery.

November 11–13: Condition good. No tetany. No longer in heat.

November 14: Slight fibrillary tremors in jaw muscles. Animal can be thrown into violent tetany by excitement or muscular exercise.

November 15–20: Condition good. No tetany. Stock diet.

November 21: Animal found to be pregnant. Diet, white bread and milk ad libitum and lactose 60 grams per day. No tetany.

November 22–December 23: Condition good. No tetany. Diet, white bread and milk ad libitum and lactose 60 grams per day. Feces liquid, odorless and acid to litmus.

December 24: Severe tetany in a.m. (Hyperpnea, tremors, salivation, tonic and clonic convulsions.) Seven hundred cubic centimeters of 0.9 per cent NaCl solution were given intravenously. Ten grams of calcium lactate were given by stomach tube. Recovery.

December 25: Four dead pups were born. These were apparently near term. Mother in good condition with no symptoms of tetany.

December 26–January 13, 1923: Condition good. No tetany. Diet, white bread and milk ad libitum, lactose 60 grams per day, calcium lactate 10 grams per day given by stomach tube.

January 14: Slight tetany. Three hundred cubic centimeters of 25 per cent lactose solution given by stomach tube. Recovery.

January 15–January 29: Condition good. No tetany. Diet, bread, milk and lactose.

January 30–February 10: Condition good. No tetany. Stock diet.

February 11: Tremors, spasticity, hyperpnea. Seven hundred cubic centimeters of 0.9 per cent NaCl solution given intravenously. Recovery.

February 12–April 29: Condition good. No tetany. Stock diet. Examination of the eyes revealed the presence of well-developed cataracts on both sides.

April 30–May 2: Dog is in heat. Observed frequently in copulation. At this time there were many severe attacks of tetany which were relieved by the administration of lactose solution by stomach tube.

May 3–June 1: Condition good. No tetany. Stock diet.

June 2: Dog is pregnant. Diet, white bread and milk ad libitum and lactose 60 grams per day.

June 3–20: Condition good. No tetany.
June 21: Condition good. No tetany.
Blood non-protein N 29.1 mgm. per 100 cc. of blood.
June 22–27: Condition good. No tetany.
June 28: Slight fibrillary contractions in the fore legs.
Blood non-protein N 28.3 mgm. per 100 cc. of blood.
June 29–July 2: Condition good. No tetany.
July 3: Condition good. No tetany.
Blood non-protein N 30 mgm. per 100 cc. of blood.
July 5: Condition good. No tetany.
Blood non-protein N 33.3 mgm. per 100 cc. of blood.
July 6: Condition good. No tetany.
At 10:00 a.m. four living pups were born. Labor was entirely normal.
No evidence of tetany or depression. The pups were normal and in good condition.
July 7–8: Mother and pups in excellent condition. No evidence of tetany.
Lactation in mother seems normal and pups are thriving.
July 9: Mother showed tremors and spasticity at 8:00 a.m.
At 9:00 a.m. blood non-protein N 30 mgm. per 100 cc. of blood.
At 12:00 m. Mother developed violent tetany (spasticity, hyperpnea, salivation, tonic and clonic convulsions). Thirty grams of calcium lactate and 500 cc. of 20 per cent lactose solution were given by stomach tube. Recovery.
July 10: Condition good. No tetany.
July 11: At 1:00 p.m. mother developed tremors, blepharospasm, and slight tonic convulsions. See figure 1.
Twenty grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube. Recovery.
Blood non-protein N 18 mgm. per 100 cc. of blood.
July 12: Mother developed marked tremors and spasticity but no convulsions.
Pups entirely normal, nursing, and growing rapidly.
Blood non-protein N of mother 32 mgm. per 100 cc. of blood.
Fifteen grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube. Recovery.
July 13: Mother developed marked tetany (spasticity, salivation, hyperpnea, tonic and clonic convulsions).
Blood non-protein N of mother 27 mgm. per 100 cc. of blood.
Fifteen grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube. Recovery.
Pups entirely normal.
July 14: Condition good No tetany.
July 15: Condition good. No tetany.
Blood non-protein N 30 mgm. per 100 cc. of blood.
Fifteen grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube.
July 16–17: Condition good. No tetany.
July 18: Condition good. No tetany.
Blood non-protein N 30.3 mgm. per 100 cc. of blood.
Fifteen grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube.
July 19: Condition good. No tetany.
Blood non-protein N 25 mgm. per 100 cc. of blood.
Fifteen grams of calcium lactate and 300 cc. of 20 per cent lactose by tube.
Pups in excellent condition.

July 20, 1923: Marked anorexia and depression. No tetany. Given 20 grams of calcium lactate and 500 cc. of 20 per cent lactose by stomach tube. No relief. Depression continued and animal died in depression at 10:00 p.m.
July 20: Autopsy done immediately. No trace of thyroid or parathyroid tissue found. Slight cataracts in both lenses (whorls with division of lens). Stomach and intestines slightly hyperemic. No ulcers found. Other organs normal in appearance.

Dog 40. Adult, female dog. Weight 11 kgm.
July 25, 1922: Diet, white bread and milk ad libitum, lactose 60 grams per day.
August 12: Feces liquid, odorless, acid to litmus. Complete thyro-parathyroidectomy.
August 13–15: Condition good. No tetany.
August 16: Mild attack of tetany. Three hundred cubic centimeters of 20 per cent lactose solution were given by stomach tybe Complete relief.
August 19: Severe attack of tetany. Five hundred cubic centimeters of Ringer's solution given intravenously. Recovery. The severe heat (outside temperature 95 to 100°F.) probably induced this severe attack of tetany.

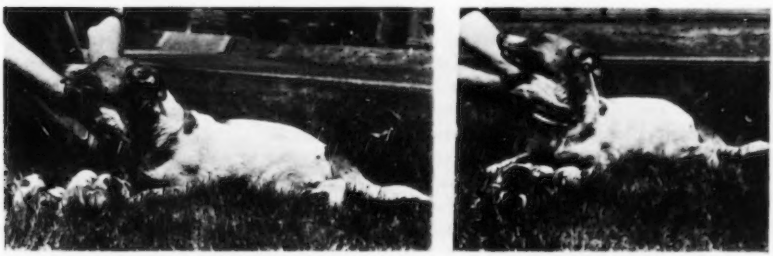

Fig. 1. Pictures taken of dog 38 on July 11, 1923, while she was in tetany brought on by lactation.

August 20–21: Condition good. No tetany.
August 22: Severe tetany. Five hundred cubic centimeters of Ringer's solution were given intravenously. Recovery.
August 23: Severe tetany. Five hundred cubic centimeters of Ringer's solution were given intravenously. The dog is in heat. This condition has most probably aggravated the tetany. Has been observed repeatedly in copulation with several different males.
August 24–31: During this period the dog has been in a precarious state. It became infected with mange. Then it became spastic, developed fibrillary tremors and some course jerking movements, passing into severe tetanic attacks of which it had three or four. These were relieved by the administration of 500 cc. of 0.9 per cent NaCl solution intravenously; 20 per cent lactose solution was given by stomach tube.
November 1–12: Condition good. No tetany.
November 13: The animal is spastic and so ataxic it cannot walk. No tremors. Five hundred cubic centimeters of 0.9 per cent NaCl solution were given intravenously. Recovery.

November 14–December 1: Condition good. No tetany.
December 1: Dog was placed on stock diet for first time since the operation.
December 2–18: Condition good. No tetany.
December 19: Slight tetany. Four hundred cubic centimeters of 20 per cent lactose solution were given by stomach tube. Recovery.
December 20: Diet, white bread and milk ad libitum, and lactose 60 grams per day.
December 21–January 11, 1923: Condition good. No tetany.
January 12–15: Dog is in heat. Has been repeatedly observed in copulation with several different males. No tetany.
January 16: Condition good. No tetany. No longer in heat. Given stock diet.
January 17–February 9: Condition good. No tetany.
February 10: Slight tetany. Placed on diet of bread, milk and lactose.
February 11: Moderately severe tetany. Three hundred cubic centimeters of 40 per cent lactose solution were given by stomach tube. Recovery.
February 12: Condition good. No tetany. A slight enopthalmos is evident and with blepharospasm becomes marked on excitement.
February 13–March 19: Dog is pregnant. The face has an anxious expression, difficult to describe but which has been repeatedly observed in the parathyroidectomized animals. Condition good. No manifest tetany.
March 20: Three living pups were delivered at 10:00 a.m. Mother showed no symptoms of tetany before, during or following labor.
March 21: Mother and pups in good condition. No tetany.
March 22: Two of the pups were crushed by the mother. The third is in good condition. The mother is depressed and refuses food.
March 23: Mother in violent tetany. Twenty grams of calcium lactate and 300 cc. of milk were given by stomach tube. Recovery.
March 24: Condition good. No tetany. Ten grams of calcium lactate were given by stomach tube. She was given another pup to nurse.
March 25: Condition good. No tetany.
March 26: Violent tetany (hyperpnea, salivation, tonic and clonic convulsions). Fifteen grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube. Recovery.
March 27–April 1: Severe and typical tetany developed daily. Recovery followed the administration of 15 grams of calcium lactate and 300 cc. of lactose solution (20 per cent) by stomach tube.
April 2–5: Condition good. No tetany. Ate voluntarily of milk, cornmeal and lactose.
April 6–9: Moderately severe tetany daily (spasticity, tremors, slight tonic convulsions). Administration of 15 grams of calcium lactate and 300 cc. of 20 per cent lactose by stomach tube brought relief.
April 10–April 15: Condition good. No tetany. Pups nursing and in excellent condition.
April 16: Marked depression at 8:00 a.m. (no tremors, no spasticity). The depression rapidly became more severe and death occurred at 4:00 p.m. There were no symptoms of tetany at any time. Autopsy was done immediately. No trace of thyroid or parathyroid tissue could be found on very careful search. Suspicious bits of tissue were sectioned and examined microscopically. There was a marked delay in the coagulation of the blood. Samples withdrawn in beakers remained liquid for four hours.

Dog 42. Adult, female dog. Weight 10 kgm.

November 3, 1922: Diet of white bread and milk ad libitum, and lactose 100 grams per day.

December 8: Feces liquid, odorless, acid to litmus. Complete thyro-parathyroidectomy. Thyroid glands unusually large.

December 9–17: Condition good. No symptoms of tetany. Appetite good. Bread, milk and lactose taken voluntarily.

December 18: Depression. Few slight tremors. Ten grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube. Recovery.

December 19: Condition good. No tetany Ten grams of calcium lactate were given by stomach tube.

December 20–25: Condition good. No tetany. Ten grams of calcium lactate were given daily by stomach tube.

December 26: Marked depression No tetany Seven hundred cubic centimeters of Ringer's solution were given intravenously. Three hundred cubic centimeters of 20 per cent lactose solution were given by stomach tube. Recovery.

December 27–30: Condition good. No tetany.

December 31: Slight tetany in morning. Eight hundred cubic centimeters of Ringer's solution were given intravenously. Ten grams of calcium lactate were given by stomach tube. Recovery.

January 1–2, 1923: Depression. Loss of appetite. No tetany. Three hundred cubic centimeters of 20 per cent lactose solution were given daily by stomach tube.

January 3–February 13: Condition good. No tetany.

February 14: Diet changed to the regular stock diet of bread, meat and vegetables.

February 15–May 3: Condition good. No tetany. Marked gain in weight.

May 4: Dog is in heat. Has been repeatedly observed in copulation with several different males. During and following copulation she developed marked hyperpnea, salivation, tremors, spasticity and tonic convulsions.

May 5: Condition good. No tetany. Still in heat.

May 6: Severe tetany. Fifteen grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube. Recovery.

May 7–June 1: Condition good. No tetany.

June 2: Dog is pregnant. Returned to diet of bread, milk and lactose.

June 3–20: Condition good. No tetany.

June 21: Condition good. No tetany.
Blood non-protein N 32.4 mgm. per 100 cc. of blood.

June 22–27: Condition good. No tetany.

June 28: Condition good. No tetany.
Blood non-protein N 30.5 mgm. per 100 cc. of blood

June 29–July 2: Condition good. No tetany.

July 3: Condition good. No tetany.
Blood non-protein N 30 mgm. per 100 cc. of blood.

July 4: Slight tetany (spasticity and tremors)
Blood non-protein N 49.2 mgm. per 100 cc. of blood.

July 5: Condition good. No tetany.
Blood non-protein N 50 mgm. per 100 cc. of blood.

July 6: Labor began at 1:00 p.m. Three living pups were born at 1:15 p.m. One dead pup was born at 1:30 p.m. Mother showed considerable hyperpnea but no tremors nor spasticity.

July 7: Condition of mother and pups at 8:00 a.m. very good. No tetany. At 10:00 a.m. the mother suddenly developed tonic convulsions and fell to the ground, killing one of the pups. The tonic convulsions were succeeded by clonic movements and in a very short time the attack ceased. The animal then appeared entirely normal. The convulsive phenomenon resembled a Grand Mal attack of epilepsy rather than the typical attack of tetany. Fifteen grams of calcium lactate and 300 cc. of 20 per cent Lactose's solution were given by stomach tube.

July 8-9: Condition good. No tetany.
Blood non-protein N 28.1 mgm. per 100 cc. of blood.

July 11: Slight tetany.
Blood non-protein N 29.5 mgm. per 100 cc. of blood.

July 12: Condition good. No tetany. Pups appear normal.
Blood non-protein N 37.5 mgm. per 100 cc. of blood.

July 14: Severe attack of tetany in a.m. Respiration stopped due to spasm of diaphragm. Artificial respiration induced. Fifteen grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube. Recovery.
Blood non-protein N 27.3 mgm. per 100 cc. of blood.

July 15: Slight tetany.
Blood non-protein N 22.2 mgm. per 100 cc. of blood.
Fifteen grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube. Recovery.

July 16: Condition good. No tetany.

July 17: At 10:00 a.m. when attempting to pass the stomach tube the dog became suddenly limp and fell to the ground apparently unconscious. The pulse was slow and weak and respiration slow and shallow. She lay on the floor in this comatose state for about four minutes, then suddenly recovered and in two minutes seemed entirely normal again.
Blood non-protein N 28 mgm. per 100 cc. of blood.
Fifteen grams of calcium lactate and 300 cc of 20 per cent lactose solution were given by stomach tube.

July 18: Two attacks exactly similar to the one of July 17 occurred during the day. During the attack the animal became very flaccid, and was apparently unconscious.

July 18: Blood non-protein N 28.2 mgm. per 100 cc. of blood.
Twenty grams of calcium lactate and 300 cc. of 20 per cent lactose solution were given by stomach tube.

July 19: Condition good. No tetany. Pups nursing and in good condition.
Blood non-protein N 28.6 mgm. per 100 cc. of blood.

July 20: Condition good. No tetany.
Blood non-protein N 28.6 mgm. per 100 cc. of blood.
Twenty grams calcium lactate were given in the food.

July 21: Condition good. No tetany. Twenty grams of calcium lactate given in food.

July 22: Condition poor. Marked depression. Pups were taken away and given to a separate mother. Fifteen grams of calcium lactate given by tube.

July 23: Condition good. No tetany. Fifteen grams of calcium lactate in food.

July 24: Condition good. No tetany. Fifteen grams of calcium lactate in food.

July 25: Condition good. No tetany. Blood non-protein N 26.2 mgm. per 100 cc. blood.

July 26: Condition good. No tetany. Blood non-protein N 26.4 mgm. per 100 cc. blood.
July 27: Condition good. No tetany. Blood non-protein N 26.2 mgm. per 100 cc. blood.
July 28: Condition good. No tetany. Blood non-protein N 26.0 mgm. per 100 cc. blood.
July 29–September 1: Condition good. No tetany. Diet of white bread and milk ad libitum, lactose 50 grams per day. No calcium lactate was given.
September 2: Severe attack of tetany. Fifteen grams of calcium lactate were given by stomach tube. Recovery.
September 3–October 3: Condition good. No tetany.
October 4: Violent tetany. Fifteen grams of calcium lactate given by stomach tube. Recovery.
October 5: Condition good. No tetany.
October 6: Severe tetany. Fifteen grams of calcium lactate given by stomach tube Recovery.
October 7–December 9: Condition good. No tetany. Diet, white bread and milk ad libitum.
December 11: Dog is in heat. Observed in copulation several times. At these times there was marked hyperpnea but no tetany.
December 12–20: Period of heat. During this entire time the dog was fed only white bread and milk ad libitum. Although observed repeatedly in copulation there was no manifest tetany.
December 21–January 12, 1924: Dog is pregnant. Condition good. No tetany. Diet white bread and milk ad libitum.
January 13: Condition good. No tetany. Diet bread and milk.
Blood calcium 9.73 mgm. per 100 cc. of blood
Blood non-protein N 30.7 mgm. per 100 cc. of blood.
January 14–16: Condition good. No tetany. Diet bread and milk.
January 17: Condition good. No tetany. Diet bread and milk.
Blood calcium 9.60 mgm. per 100 cc. of blood
Blood non-protein N 32.4 mgm. per 100 cc. of blood
January 18–20: Condition good. No tetany. Diet bread and milk.
January 21: Condition good. No tetany. Diet bread and milk.
Blood calcium 9.70 mgm. per 100 cc. of blood.
Blood non-protein N 31.91 mgm. per 100 cc. of blood.
January 23: Condition good. No tetany. Diet bread and milk.
Blood calcium 9.90 mgm. per 100 cc. of blood.
Blood non-protein N 30.71 mgm. per 100 cc. of blood.
January 25: Condition good. No tetany. Diet bread and milk.
Blood calcium 9.30 mgm. per 100 cc. of blood.
Blood non-protein N 34 50 mgm. per 100 cc. of blood.
January 28: Condition good. No tetany. Diet bread and milk.
Blood calcium 9.31 mgm. per 100 cc. of blood.
Blood non-protein N 34 50 mgm. per 100 cc. of blood.
February 1: Condition good. No tetany. Diet bread and milk.
Blood calcium 10.40 mgm. per 100 cc. of blood.
Blood non-protein N 35.00 mgm. per 100 cc. of blood.
February 4: Violent attack of tetany at 9:00 a.m. (hyperpnea, salivation, spasticity, tonic and clonic convulsions). On attempting to pass a stomach tube the dog became suddenly very flaccid, fell in collapse and appeared about to die.

After remaining in this moribund state for six minutes it suddenly recovered. It was then given 10 grams of calcium lactate in solution by stomach tube. Three hours later blood was withdrawn for examination.

Blood calcium 9.90 mgm. per 100 cc of blood.

Blood non-protein N 64.77 mgm. per 100 cc. of blood.

February 5–7: Condition good. No tetany. Diet bread and milk.

February 8: Condition good. No tetany. Diet bread and milk.

Blood calcium 9.70 mgm. per 100 cc. of blood.

Blood non-protein N 34.50 mgm. per 100 cc. of blood.

February 9–10: Condition good. No tetany. Diet bread and milk.

February 11: Found in severe tetany in the morning. Died before any treatment could be given. Blood withdrawn after death showed the following figures:

Blood calcium 9.30 mgm per 100 cc. of blood.

Blood non-protein N 20.00 mgm. per 100 cc. of blood.

Autopsy performed about five hours after death. The blood was still liquid in the larger vessels. No suspicious glandular tissue was found at the site of the thyroids, but one gland (4 × 4 × 2 mm.) was found in the lower part of the neck anterior and two smaller glands were found in the mediastinum along the aorta and pulmonary artery which on microscopic examination proved to be thyroid tissue. No trace of parathyroid tissue was found in these or in other tissues examined microscopically. Cataracts were found in both eyes. The liver was very hyperemic and contained many hemorrhagic areas. The kidneys were hyperemic. The adrenals were much larger than normal and showed a hemorrhagic zone between the cortex and medulla. The thymus was present, appeared normal and no trace of parathyroid tissue was found on examination of a number of sections. The uterus was gravid and five pups apparently near term were removed. Microscopic examination of the thyroids of the fetuses was made.

Parathyroidectomy in pregnant dogs in which intestinal putrefaction has been checked. If male or non-pregnant female dogs are fed a diet consisting exclusively of white bread and milk ad libitum and lactose from 60 to 100 grams per day, bacterial proteolysis in the intestines will be very greatly reduced and the parathyroids may be removed from these dogs without causing the usual tetany or depression and death. In the four following experiments pregnant dogs were given the above described special diet and after examination of the feces indicated that the putrefactive flora had been largely supplanted by aciduric organisms, the thyroid and parathyroid glands were extirpated. The results are given in the following protocols.

Dog 29. Adult, female dog. Weight 10 kgm.

April 18, 1922: Diet, white bread and milk ad libitum and lactose 60 grams per day. *The animal is pregnant.*

April 25: Feces liquid, odorless and acid to litmus. Complete thyro-parathyroidectomy.

April 26: In morning the animal was considerably depressed and showed slight clonic convulsions in the forelegs. No hyperpnea. Anorexia. Two hundred cubic centimeters of milk and 400 cc. of lactose solution (25 per cent) were given by stomach tube. At 8:00 p.m. there was slight tetany. Six

hundred cubic centimeters of Ringer's solution were given intravenously with a pump.

April 27: No evidence of tetany. Anorexia. Eight hundred cubic centimeters of milk and 600 cc. of lactose solution (25 per cent) were given by stomach tube.

April 28: No evidence of tetany. Anorexia. Eleven hundred cubic centimeters of milk and 600 cc. of lactose solution (25 per cent) were given during the day by stomach tube.

April 29: The animal was found dead in the morning. The body was very stiff and in marked opisthotonus. One living and one dead pup were found in the cage. There were three dead pups in utero. No trace of thyroid tissue could be found in the mother.

Dog 37. Adult female dog. Weight 12 kgm.

July 28, 1922: The animal is about five weeks pregnant Diet, white bread and milk ad libitum and lactose 60 grams per day

August 2: Feces liquid, odorless, acid to litmus. Complete thyro-parathyroidectomy.

August 3: Marked depression and anorexia. No tetany. Five cubic centimeters of 25 per cent lactose solution were given by stomach tube.

August 4: Marked depression and anorexia. No tetany. Five hundred cubic centimeters of 25 per cent lactose solution given by tube.

August 5: Found dead in cage in morning. The body was rigid, the legs extended, and it was probable that the dog died in tetany. At autopsy no trace of thyroid or parathyroid tissue was found.

Dog 48. Adult female dog. Weight 16 kgm.

March 1, 1923: Animal is pregnant. Diet, white bread and milk ad libitum, lactose 60 grams per day.

March 12: Feces liquid, odorless, acid to litmus. Complete thyro-parathyroidectomy.

March 13: Condition good. No tetany. Three hundred cubic centimeters of 20 per cent lactose solution were given by stomach tube.

March 14: Slight tremors, no spasticity. Four hundred cubic centimeters of 20 per cent lactose solution were given by stomach tube. Recovery.

March 15: Condition good. No tetany. Ate bread and milk voluntarily.

March 16: Violent attack of tetany and death at 8:00 a.m.

Dog 36. Small female dog; weight 9 kgm.

July 15, 1922: Animal is about six weeks pregnant. Diet, white bread and milk ad libitum and lactose 60 grams per day.

July 21: Feces liquid, odorless, acid to litmus; fecal bacteria predominantly aciduric. Complete thyro-parathyroidectomy.

July 22: Animal was in tetany (salivation, hyperpnea, clonic convulsions). Seven hundred cubic centimeters of 20 per cent lactose solution given by stomach tube in morning; 600 cc. of Ringer's solution given intravenously in afternoon. Recovery.

July 23: No tetany. Condition fairly good. Four hundred cubic centimeters of 20 per cent lactose solution given by stomach tube.

July 24: Slight fibrillary tremors in temporal muscles. No convulsions. Voluntarily drank some milk and lactose solution.

July 25: Slight depression. Few transient tremors. Four hundred cubic centimeters of 20 per cent lactose solution given by stomach tube.

July 26: 8:00 a.m. Slight fibrillary tremors in temporal muscles. 9:00 a.m. Marked hyperpnea, spasticity, tonic and clonic convulsions. Five hundred cubic centimeters of Ringer's solution plus 1 gram of calcium lactate given intravenously. 10:00 a.m. Convulsions subsided. Four dead pups were born. No tetany.

July 27 and 28: No tetany. Slight depression. Refused food.

July 29: Violent tetany (tonic and clonic convulsions). Five hundred cubic centimeters Ringer's solution given intravenously. Five hundred cubic centimeters of 20 per cent lactose solution given by stomach tube. Tetany relieved.

July 30–November 3: Condition good. No tetany or depression.

It will be noted that whereas pregnant dogs on ordinary diets die in severe tetany within thirty-six hours after complete parathyroidectomy, these animals on the special diets survived four, three and four days respectively, and that one was carried through the period of pregnancy by additional measures.

Comment. The clinical observations that menstruation may transform a latent idiopathic tetany into an active form and the experimental findings in the present study and by Luckhardt (7) of the development of acute tetany in completely parathyroidectomized dogs at oestrus are significant with regard to the pathogenesis of both forms of tetany. It is very probable that the metabolic changes during oestrus in the dog are similar to those occurring during menstruation in primates. The pathogenesis of idiopathic tetany in man is perhaps unsettled but the fact that it is affected in the same direction by menstruation is additional evidence that a physiological parathyroid deficiency is probably the chief underlying factor.

It is difficult to understand why oestrus should produce such a striking effect on the recovered parathyroidectomized dog. Until the period of heat the animal appears normal in every way. A number of basal metabolic rate determinations on these animals have been made and they fall within the range of the normal. This is surprising in view of the fact that the thyroids have also been completely extirpated and no trace of accessory tissue has been found in a number of the animals coming to autopsy. According to the accurate and prolonged study by Kunde (8) on the basal metabolism of normal dogs under various conditions, the period of rut either has no effect on the basal metabolic rate or decreases it. Blunt (9) has found that menstruation in normal women likewise has little or no constant effect on the basal metabolic rate. It is evident therefore that if the tetany poisons are increased during oestrus, and this seems most probable, these poisons are not of endogenous origin. It has been demonstrated that the chief source of the poisons responsible for parathyroid tetany is the gastro-intestinal tract, but there is no evidence

indicating an increased production or absorption of these at oestrus. In fact in several cases in which tetany developed at oestrus the production of poisons in the intestines had been checked by diet. It is quite possible however that the changes in the uterine mucosa during heat may result in the local production of increased toxic protein derivatives and their absorption into the blood stream. In such event the tetany of oestrus would fall in line with that of pregnancy, namely, an intoxication coming in large part from the uterus.

The immediate and complete relief of the tetany of oestrus obtained by the intravenous injection of large amounts of Ringer's solution (500 to 1200 cc. once or twice daily) supports the view that this tetany is also an intoxication. It cannot be the action of the calcium of the Ringer's solution that is solely responsible for the relief since this may also be obtained with somewhat larger amounts of 0.9 per cent NaCl solution. The latter should dilute the plasma calcium and increase the tetany if the calcium deprivation theory were correct. That the tetany of oestrus is aggravated by the absorption of putrefaction products in the intestinal tract is indicated by the fact that such tetany is worse in dogs on stock or meat diets and may be absent entirely when bacterial proteolysis in the intestine is checked. The oral administration of calcium lactate in sufficient amounts will also prevent and control the tetany of oestrus. The mechanism of its action has not yet been determined.

The direct relation between pregnancy and tetany is a striking phenomenon and one of great practical significance. In the analysis of this condition it is necessary to consider that the fetus is drawing on the maternal organism for all materials for its growth and maintenance and that also a great many substances are constantly passing from the pregnant uterus into the maternal blood stream. Some of these substances come from the fetus and others from the maternal and fetal placenta. It is plain that amongst other materials, the fetus must obtain calcium from the mother and if it can be demonstrated that this calcium drain actually lowers the blood calcium of the mother, considerable support to the calcium deficiency hypothesis of tetany might be adduced. It would however be very surprising to find that the mechanism regulating the blood calcium in the mother is so easily upset and is inadequate to what must be a very slow and gradual withdrawal of this element. In view of what has been demonstrated regarding the very efficient mechanisms for regulating many of the other physiological constants, a calcium deficiency (decreased concentration of calcium in the blood) would appear very improbable. In one experiment (dog 42) in which the concentration of blood calcium was carefully followed during a period of pregnancy, it was found that the concentration was the same as in normal dogs and that there was no variation immediately preceding the fatal attack of tetany.

In a like manner it does not seem probable that the very gradual withdrawal of other substances normally present in definite concentration in the maternal blood could so alter that constant as to produce disease.

Moreover the fact that the tetany of parathyroidectomized pregnant dogs may be controlled and prevented by the intravenous injection of Ringer's solution, as shown by Luckhardt and Rosenbloom (10), suggests that this form of tetany is also due to an intoxication. In our series of female dogs that were preserved by dietary treatment after parathyroidectomy and who subsequently became pregnant and developed tetany, it was found that all symptoms of tetany could be controlled by the intravenous injection of 0.9 per cent NaCl solution. It is difficult to see how this fact can be explained on any other basis than that the diuresis induced was sufficient to remove the poisons in the blood responsible for the tetany. It is certain that calcium played no part in the alleviation of this tetany.

A diet of bread, milk and lactose, which will prevent the onset of tetany in parathyroidectomized male or non-pregnant female dogs, does not suffice to protect pregnant animals. The tetany is delayed, but occurs and is invariably fatal unless additional measures are adopted. This fact suggests that, during pregnancy, poisons capable of causing tetany or depression are being absorbed from some other source as well as the intestinal tract. The observation that the tetany occurring during pregnancy is relieved when the uterus is emptied, makes it practically certain that the pregnant uterus is the chief additional source for this toxemia. The occurrence of tetany on December 24 in dog 38 during the latter part of pregnancy, in spite of the fact that all putrefaction in the intestines had been checked, strongly supports this view. It appears then that during pregnancy the detoxicating mechanism of the body is under an additional strain, having to deal with toxic protein derivatives from both the uterus and the gastro-intestinal tract as well as perhaps other sources less definitely known. Since the parathyroid glands in some way form a part of this detoxicating mechanism, it is not surprising to find that pregnant animals become more rapidly intoxicated following parathyroidectomy than others and that this intoxication is only partly controlled by checking bacterial proteolysis in the intestines.

Perhaps the strongest support to the calcium deprivation theory of tetany is to be obtained from the phenomenon of lactation tetany. The tetany recurring in the parathyroidectomized dog during lactation may be more severe and difficult to control than either the tetany of oestrus or pregnancy. It can be relieved by removing the pups and stopping the suckling or it can be made worse by increasing the litter to be fed by the lactating mother. It seems quite definite that the withdrawal of milk is an important exciting factor in this type of tetany. In view of this it

is significant that the tetany can be relieved and prevented by the administration of calcium lactate by mouth in relatively large amounts (20 to 40 grams per day). This relief can be obtained even though the suckling be continued. The fact however that the tetany can also be relieved and controlled by the intravenous injection of large amounts of either Ringer's solution or 0.9 per cent NaCl solution argues against the physiologic specificity of calcium action. The calcium does not seem to play an essential rôle.

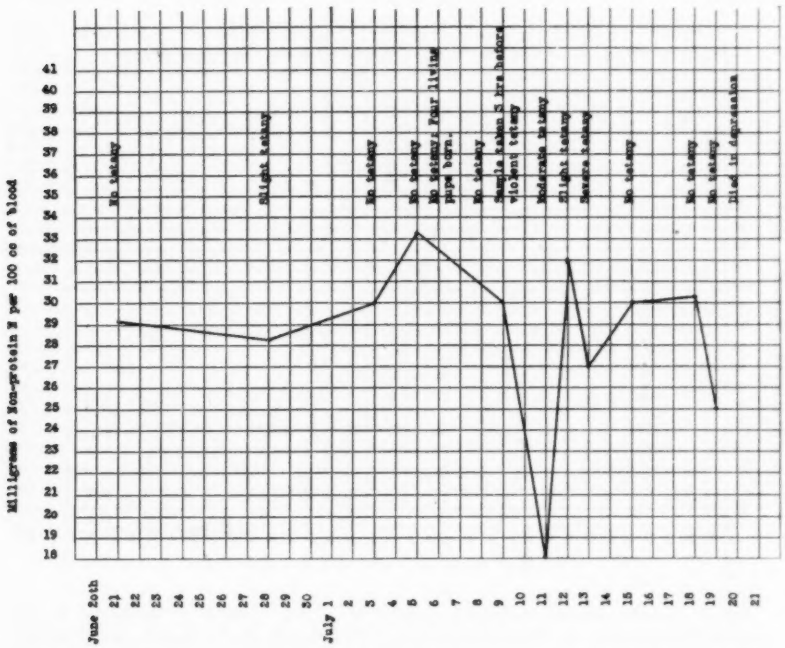

Fig. 2. Graphic record of dog 38 showing the non-protein N of the blood during periods of pregnancy and lactation tetany. The dog was probably impregnated May 1, 1923. Thyro-parathyroidectomy done August 2, 1922.

Non-protein nitrogen and calcium in the blood during the tetany of pregnancy and lactation. Examinations were made of the non-protein nitrogen of the blood during a period of pregnancy and lactation in dog 38. The details of the findings are given in the protocol and may be seen by inspecting the graphic record in figure 2. In this case there is no evidence of significant variation in the non-protein nitrogen of the blood in either the tetany of pregnancy or lactation. The results fall within the variations found in normal dogs.

In dog 42 the non-protein nitrogen of the blood was determined during one pregnancy and lactation and the non-protein nitrogen and calcium in the blood during a second pregnancy and lactation. (See protocol of dog 42.) The results in the first case (graphic record, fig. 3) indicate that there is no constant increase or decrease in the non-protein nitrogen during the tetany of pregnancy or lactation. As in dog 38, there was a marked increase just before delivery but this increase was not uniformly associated with tetany. Graphic record, figure 4, shows the non-protein nitrogen and calcium in the blood in dog 42 during a second period of pregnancy

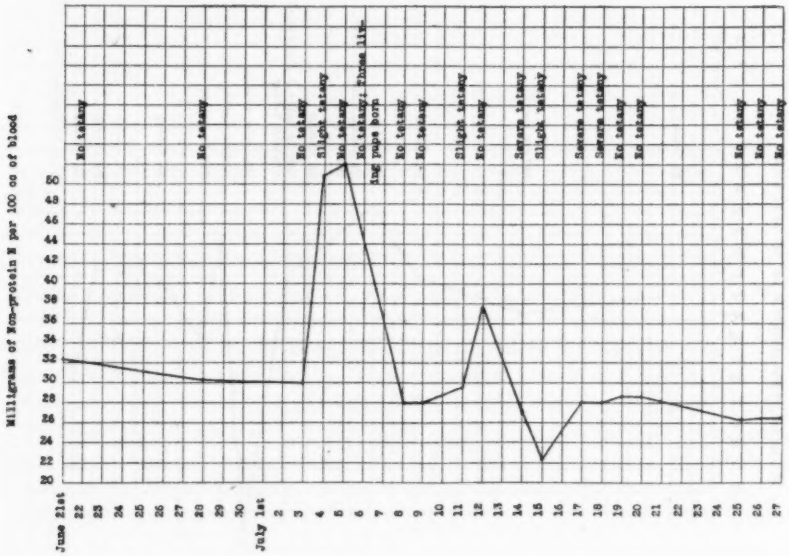

Fig. 3. Graphic record of dog 42 showing the non-protein N of the blood during periods of pregnancy and lactation tetany. Dog was probably impregnated May 5, 1923. Thyro-parathyroidectomy done December 8, 1922.

and lactation. It will be noted from inspection of the curve that there was a marked increase in the non-protein nitrogen in the blood three hours after a severe attack of pregnancy tetany. This is however more probably the result of the tetany than the cause. The blood taken after the death of the animal from pregnancy tetany showed the lowest concentration of non-protein nitrogen found at any time. The blood calcium was determined by the method of G. W. Clark (11). At no time did the concentration of blood calcium fall below 9.0 mgm. per 100 cc. of blood and three hours after a severe attack of pregnancy tetany it was 9.9 mgm. Although it is not possible to draw conclusions from one experiment, the

findings in this case do not indicate that a deficiency of blood calcium occurs during pregnancy or that the concentration of blood calcium bears any relation to the tetany of pregnancy.

The parathyroid glands and eclampsia. There are many suggestions in the experimental literature of a possible etiological relationship between parathyroid deficiency and the various toxemias of pregnancy. Vassale (12), Massaglia (13) and others who have noted the recurrence of tetany in partially parathyroidectomized animals during pregnancy have emphasized the similarity of this disease and clinical eclampsia. In our own experiments the development of severe and fatal convulsions during the

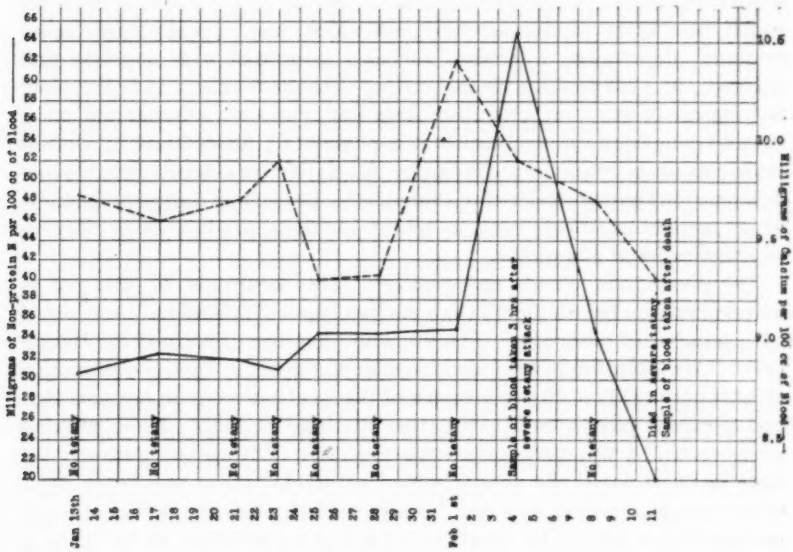

Fig. 4. Graphic record of dog 42 showing the non-protein N and calcium of the blood during periods of pregnancy and lactation tetany. Dog was probably impregnated December 12, 1923. Thyro-parathyroidectomy done December 8, 1922.

latter part of pregnancy in completely parathyroidectomized dogs who have previously appeared normal has certainly been very striking. The relation between eclampsia and the toxemia occurring during pregnancy in the parathyroidectomized dog may be summarized as follows:

1. In both the experimental animal and in man the best evidence available indicates that in each case there exists a toxemia arising from the pregnant uterus and caused by toxic protein derivatives.

2. Both diseases are characterized by the appearance of tonic and clonic convulsions usually during the latter part of pregnancy or immediately after delivery.

3. Both diseases are relieved and approximately to the same degree by emptying the uterus.

4. In both conditions the non-protein nitrogen concentration of the blood remains within normal limits or is slightly increased.

5. Both diseases are associated with a persistent albuminuria of varying degree.

It is quite true that idiopathic tetany in man presents a definite clinical syndrome, made worse by pregnancy, and which can be readily differentiated from true eclampsia. The removal of the parathyroid glands however, does not always cause tetany, although this is the most constant syndrome seen in carnivorous animals. In the dog a toxemia develops which most often produces tetany but which may in many cases cause profound depression, a gradual cachexia associated with anorexia and diarrhea, or a marked ataxia and stupor without tetany. It is therefore not logical to say that eclampsia does not represent a functional parathyroid deficiency simply because it is possible to differentiate by clinical signs eclampsia from typical tetany. A relative or absolute parathyroid deficiency in the dog renders the animal liable to toxemia during pregnancy, which usually manifests itself as tetany but may produce other nervous or constitutional symptoms.

The parathyroid glands and cataract. The occurrence of cataract in individuals who have suffered from various types of tetany has been noted by a number of observers. The literature has been reviewed by Barker (14). Our attention to this phenomenon was directed by the observation of Luckhardt (15) of the occurrence of bilateral cataracts in animals that had survived parathyroid extirpation for long periods. These animals were treated by the daily intravenous injection of Ringer's solution. We have likewise noted the appearance of cataracts in six completely parathyroidectomized dogs that had been carried through the period of acute post-operative tetany by checking intestinal putrefaction or by the oral administration of calcium lactate. The first evidence of disturbance in the lens may usually be seen from three to six months after the operation. The division of the lens becomes visible and many opaque spots (punctate cataract) appear throughout the lens and on the posterior capsule. The condition is progressive and after eighteen to twenty-four months may lead to complete opacity and partial blindness.

From the preceding analysis it appears probable that the cataract is the result of a continuous low grade toxemia, due to the experimentally produced defect in the detoxicating mechanism of the body, which affects the lens and perhaps also many other structures where slight changes are not so readily observed.

SUMMARY

Tetany and the oestrus cycle. Regarding the relation between tetany and the periodic sex activity or oestrus, the following observations may be recorded:

1. The complete absence of the thyroid and parathyroid glands does not prevent the regular appearance of oestrus in dogs.

2. Slight parathyroid intoxication may prevent the development of oestrus in dogs for the first one or two periods after the glands have been removed.

3. Coincident with the appearance of oestrus, the dog, from which the thyroid and parathyroid glands have been long absent and which has been for many months in excellent condition and free from tetany, may at this time develop the typical syndrome of acute parathyroid tetany in all degrees of severity. Unless adequately treated the great majority of these animals die in either acute tetany or severe depression.

4. The tetany of oestrus may be entirely controlled by *a*, the intravenous injection of large quantities of Ringer's solution; *b*, the intravenous injection of large quantities of 0.9 per cent NaCl solution; *c*, the administration of large quantities of calcium lactate (15 to 30 grams per day) by mouth; or *d*, by the adoption of a bread and milk diet and the administration of large quantities of lactose solution (300 to 500 cc. of a 20 per cent solution) by stomach tube.

Tetany and pregnancy. With regard to the relation between pregnancy and tetany the observations may be summarized as follows:

1. The complete absence of the thyroid and parathyroid glands does not prevent pregnancy in the dog. A thyro-parathyroidectomized female may be impregnanted by a thyro-parathyroidectomized male.

2. During the latter half of pregnancy, the dog, from which the thyroid and parathyroid glands have been absent for many months and which is apparently in good condition, may suddenly develop the syndrome of acute parathyroid tetany in all degrees of severity. This tetany is more acute and more rapidly fatal than that which appears in non-pregnant animals upon parathyroid extirpation.

3. An attack of tetany, not immediately fatal to the mother, usually results in the death of the fetuses and is followed by abortion. The termination of pregnancy has a beneficial effect on the tetany. It is probable that the fetuses are killed by asphyxia rather than by the tetany intoxication.

4. Tetany which develops in the completely parathyroidectomized dog during pregnancy may be controlled by *a*, the intravenous injection of large amounts of Ringer's solution; *b*, the intravenous injection of large amounts of 0.9 per cent NaCl solution; and *c*, the feeding of large amounts of calcium lactate (15 to 30 grams daily) by stomach tube.

5. The completely thyro-parathyroidectomized dog may become pregnant and deliver normal pups at term, without showing tetany or depression at any time if kept on a diet of white bread and milk ad libitum and lactose (60 grams per day) during the entire pregnancy.

6. The special diet which will prevent intestinal putrefaction in the dog and which suffices to prevent parathyroid tetany in male and non-pregnant female dogs will delay and lessen the severity of tetany following parathyroidectomy in pregnant animals but will not prevent it nor permit the animals to survive.

7. The non-protein nitrogen of the blood and the calcium concentration in the blood of thyro-parathyroidectomized dogs remains within normal limits and shows no constant variation coincident with the development of tetany during pregnancy or lactation.

Tetany and lactation. With regard to the relation between lactation and tetany the observations may be summarized as follows:

1. The complete absence of the thyroid and parathyroid glands does not prevent the hypertrophy of the mammary glands during pregnancy and the secretion of milk after delivery.

2. If tetany in the parathyroidectomized dog has been controlled during the entire period of pregnancy, normal pups may be delivered and these thrive on the mother's milk. The pups do not develop tetany or depression.

3. During lactation the parathyroidectomized mother may develop the typical syndrome of acute parathyroid tetany in all degrees of severity. If lactation continues and no special effort be made to control the tetany, the majority of these animals will die in tetany or depression.

4. The tetany of lactation can be controlled or prevented by *a*, the intravenous injection of large amounts of Ringer's solution daily; *b*, the intravenous injection of large amounts of 0.9 per cent NaCl solution daily; or *c*, the administration of large amounts of calcium lactate daily by stomach tube.

5. The severity of the tetany depends in part at least upon the amount of milk withdrawn. It can be relieved entirely by removing all the pups or partially by removing all but one or two. Conversely the tetany can be made more severe by giving the mother several additional pups to suckle.

Tetany and cataract. Dogs that have been completely thyro-parathyroidectomized and have been preserved by various treatments develop bilateral cataracts. The cataract may appear as early as three months after the operation and is usually rapidly progressive leading eventually to complete opacity of the lens.

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EFFECT OF THYROXIN UPON THE BLOOD SUGAR OF NORMAL AND THYROIDECTOMIZED SHEEP

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The early researche[illegible]J. R. Macleod, Banting, and their associates upon insulin and its effe[illegible], laid down the general lines for many types of investigation.

Eadie and Macleod reported experiments on the antagonistic effects of adrenalin and insulin (1). The decrease of sugar tolerance which is produced by administration of thyroid gland or thyroxin and the increase produced by thyroidectomy, and the parallel phenomena observed clinically in hyperthyroidism and hypothyroidism, suggest an investigation of the relations of thyroxin and insulin in the regulation of the blood sugar. Some preliminary observations have already been reported (2).

The following studies were undertaken:

1. The determination of the blood sugar changes in normal and thyroidectomized subjects after repeated administration of thyroxin (or thyroid extract).

2. The determination of the blood sugar changes in normal and thyroidectomized subjects after administration of insulin.

3. The determination of the manner in which the simultaneous presence of insulin and thyroxin modifies the blood sugar changes due to each.

The present paper deals with the first problem named.

Alimentary hyperglycemia has been found by many observers to be characteristic of the hyperthyroid state, but there seem to be no definite or conclusive comparisons of the fasting blood sugar in the hyperthyroid and normal states. For our purposes, moreover, the reactions of our own material to thyroxin had to be studied in preparation for the other phases of the problem.

Our experimental material consists of a flock of normal and thyroidectomized sheep maintained for six years by Dr. Sutherland Simpson and used for a variety of research problems. Growth and other data have been kept for each sheep by Doctor Simpson, so that each animal's history is fairly well known and helps to interpret the experimental data.

Through frequent handling in the course of various routine observations upon them, the animals have become tame and submit quietly to the repeated sampling of blood necessary in the course of these experiments.[1]

The blood sugar values in normal and thyroidectomized sheep have been reported (3). Continued tests confirmed the values reported originally for the normal sheep. The thyroidectomized sheep as a group show less constancy. The age at which the operation was performed and the possible presence of accessory tissue are among the factors which may be of importance in this connection. The likelihood of the appearance of secondary effects of thyroidectomy, some of which may affect the blood sugar, seems to be an important factor. The primary effect is to produce hypoglycemia. However, since our first report of the case of a cretin with hyperglycemia (4), we have observed several other cases. In the case reported the pancreas seemed normal, but the adrenals were greatly hypertrophied. The other cases of hyperglycemia in cretins are awaiting their post-mortems for an explanation.

Only a limited number of cretins have been available each season, and the progress of the study of the differences within this group is therefore necessarily slow.

The basal sugar values ("fasting") may be said to be 60 ± 5 mgm. per 100 cc. for the normal sheep and 45 ± 5 for the cretins. Individual sheep, apparently normal, may show higher or lower values occasionally, or even consistently. But the values given will apply to most sheep, under the conditions which define the term "fasting" as used in this paper. The blood sugars of each sheep fluctuate within a narrower range. Attempts are being made to minimize these fluctuations. The difficulties attending these efforts are probably due in part to the peculiarities of ruminating animals.

In the meantime these fluctuations are discounted by reference to normal controls, it having been observed that in most normal sheep the changes on a given day are in the same direction, and of about the same magnitude (see particularly the earlier portions of the curves, representing the results of the preliminary tests, figs. 4, 5 and 6).

Experiment 1. Our object was to ascertain the effect of thyroxin upon the blood sugar of normal and thyroidectomized sheep while recently ingested food was being actively absorbed, and to compare it with the

[1] Although we cannot tell, on the basis of the few data which we have obtained incidentally, of what importance emotional hyperglycemia may be in sheep generally, our data on our own material show it to be of no significance under the conditions of our experiments. Repeated pricking of the skin preliminary to the withdrawal of the sample did not affect the sugar values materially. No sudden variations were observed in the sugar content of the blood when samples were taken at intervals during the day; changes, if any, developed gradually. Changes that were observed from day to day were parallel for all or almost all sheep, and are discussed elsewhere.

effect upon the "fasting" blood sugar. Our material consisted of sheep and lambs which were also being used in other experiments, and which were receiving thyroxin, thyroid extract and sodium iodide. We therefore had the opportunity to compare the effects of the last two substances with the effect of thyroxin.

Five cretin sheep and three normal lambs of about the same weight as the cretins were observed. Two normal sheep, of the same age as the cretins (twin sisters of two of the cretins), were used as controls.

A summary of the records of the sheep and of their treatment follows:

The normal and cretin sheep were about eighteen months old. The cretins had had their thyroids removed at the age of about one month. The cretins are designated by the letter O (operated), the normal sheep by the letter N. The normal lambs were about six months old. They were designated by the letter W.

2-N (weighing 132 pounds) and 5-N (126 pounds) were controls, receiving no treatment.

1-O (72 pounds), 4-O (55 pounds), 11-O (77 pounds), and 1-W (77 pounds) received 0.25 mgm. of thyroxin subcutaneously.

2-O (61 pounds) and 4-W (64 pounds) received 0.25 gram of thyroid extract in gelatin capsules.

5-O (83 pounds) and 3-W (64 pounds) received 0.5 gram of sodium iodide in gelatin capsules.

The drugs were administered every other day.

1-O was killed by stray dogs and 11-O died before the conclusion of the experiment. The results with the other cretins, while representing few cases, are reported at this time because certain fundamental observations are not likely to be contradicted in further tests, which must, however, be delayed until similar material is again available for this phase of the subject.

All the sheep were together under similar external conditions.

The first period of observations continued from October 15 to November 5, while the grazing was still good. Blood was taken at about 3 p.m. On November 30, just before the sheep were transferred to winter quarters for lack of nourishment in pasture, a determination was made at about 3 p.m.

"Fasting" blood sugars, for comparison with results of the preceding tests, were determined during the second period, after the sheep had been transferred. The sheep were fed hay and a grain mixture about 4 to 5 p.m. the day preceding the tests, allowing an interval of about eighteen hours after the last meal. That was probably a sufficiently close approach to "fasting," even in ruminants.[2] A longer interval was not feasible.

[2] The term "fasting" is used in this paper only as here defined.

The administration of drugs began on October 22, after several preliminary determinations of blood sugar, extending over about a week, had been made on the normal and cretin sheep. Only one preliminary test of the blood sugar of the lambs had been performed. Blood was taken from the jugular vein, and the sugar of the whole blood was determined by the method of Folin and Wu.

The tests were continued, at intervals, until November 5 (fourteen days after beginning of treatment).

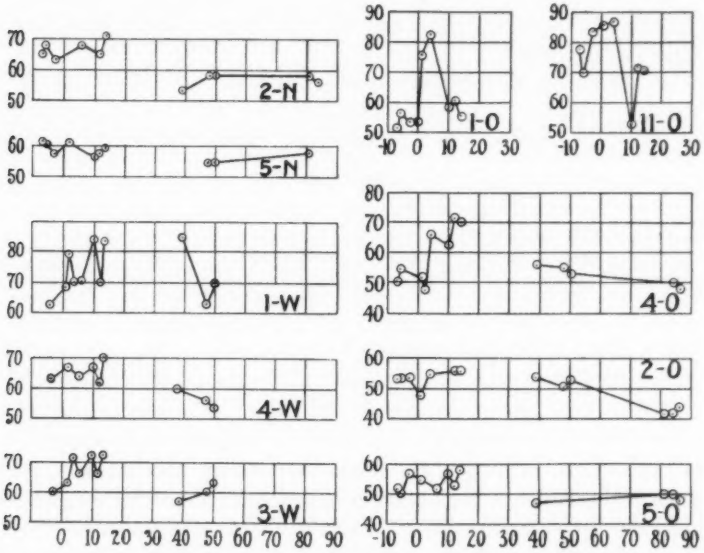

Fig. 1

Fig. 2

Fig. 1. Normal ewes. 2-N and 5-N, controls, receiving no treatment. 1-W, treated with thyroxin; 4-W, with thyroid extract; 3-W, with sodium iodide.

Fig. 2. Cretin ewes. 1-O, 11-O and 4-O received thyroxin; 2-O, thyroid extract; 5-O, sodium iodide.

Ordinates, milligrams of sugar in 100 cc.; abscissas, days after treatment began.

It will be observed (figs. 1 and 2) that during this period the most marked increase of blood sugar occurred in the cretins 1-O and 4-O and in the normal lamb 1-W—all treated with thyroxin. Cretin 11-O, also under thyroxin treatment, behaved atypically both in the preliminary tests and under treatment, probably due to secondary effects of hypothyroidism.

The effect was immediate in 1-O, being very pronounced one day after the first injection. In 4-O the effect appeared on the fourth day after the first injection but was not present on the first or second days. 1-W showed a slight increase one day after the first injection—from 63 mgm. to 69 mgm. per 100 cc. One day later the sugar was up to 79 mgm.

The higher blood sugar values were not maintained at a constant level. They fluctuated within a wider range than in the controls, remaining however well above the initial values.

It is interesting to note the correlation in some sheep between the fluctuations of the blood sugar and the fluctuations of the pulse rate and of muscular activity.

It is well known that cretins show a relatively low pulse rate and are relatively sluggish. Dr. Sutherland Simpson has been observing for some years the pulse rates of normal and cretin sheep kept under the same conditions. He has also recorded similar observations for the animals in this series. Liddell and Simpson have measured the variations in muscular activity from day to day in the animals of this series (5).

TABLE 1

DAYS AFTER TREATMENT BEGAN	1-W		4-O		
	Sugar in 100 cc.	Pulse	Sugar in 100 cc.	Pulse	Activity, steps per day
	m.m.		*m.m.*		
1	69	116	52	120	19041
2	79	130	48	110	12814
4	71	112	66	157	29698
6	71	118			
10	84	136	63	153	24908

In an attempt to find an explanation of the variations of the blood sugar, the present author has examined the pulse rate and activity records (yet unpublished). In 1-W the high sugar values corresponded with *large* increases in the pulse rate, and in 4-O also with increased activity. (See table 1.) We do not believe that the high sugars and pulse rates are due in any great measure to the increased daily activity as such, but rather that all these are, in part at least, independent effects of thyroxin administration. These correlations were observed consistently only in 1-W and 4-O, and in those only during the first ten days of the treatment.

11-O is the atypical case previously reported (4). It showed a high pulse rate and very high but fluctuating blood sugar during the preliminary tests. One day and four days after the administration of thyroxin the blood sugar rose a little higher still. On the 10th day a very *low* value was observed, accompanied by a sharp *rise* in the pulse rate. A recovery to higher blood sugar then occurred. About two weeks later the sheep died.

On post-mortem examination the pancreas appeared normal, but the adrenals were greatly hypertrophied, particularly the cortex.

The effect of the thyroid extract and of sodium iodide, in the doses administered, was not great. The blood sugar curves of 2-O and 5-O (fig. 2) and of 3-W and 4-W (fig. 1) show fluctuations which are not appreciably greater than those observed in the controls. The pulse rate remained constant and relatively low.

On November 30 (thirty-nine days after the first injection), the treatment having continued in the interval, but the amount of food in pasture having become inadequate, a blood sugar determination was made at the same time of the day as in the preceding tests, namely, at about 3 p.m.

The blood sugars proved markedly lower than on November 5 in all sheep with the exception of 2-O and 1-W. The general effect may be attributed to inadequate nutrition. The maintenance of the blood sugar in 2-O may be due to the slower depletion of the glycogen store by thyroid extract. In 1-W the occurrence of a high sugar value on November 30 is probably to be explained by the very good condition of the lamb at the beginning of the experiment. That the sugar level of 1-W was not uniformly high is suggested by the low figure on December 9. Were more points present, the curve would probably have resembled those in figures 4, 5 and 6, showing the effect of thyroxin by a higher average blood sugar concentration, while the figures on different days may vary widely.

On December 1 (40th day) the sheep were transferred from pasture to winter quarters, and fasting blood sugars were determined on December 9 (48th day) and 11 (50th day). The explanation of the low blood sugars of November 30 as due to inadequate feeding is supported by the nearly identical values obtained on the "fasting" blood. Similar values were also obtained on January 6 (76th day), 9 (79th day), and 11 (81st day) when the "fasting" blood sugar was again determined.

The administration of drugs had been discontinued between December 23 (62d day) and January 2 (72d day). This seemed to be without marked effect on the blood sugars of January 6, 9 and 11, except in 2-O, in which these values were much lower than a month before (about 40 mgm. as compared with 53).

The results of experiment 1 indicate that definite increases in the blood sugar of both normal and thyroidectomized sheep can be produced (with the dosages used) most easily by thyroxin. Fluctuations of the blood sugar under apparently similar dietary conditions are characteristic. Early in the test, that is, for about two weeks after the effect of thyroxin had been established (the latent period was variable, about one to four days) the average blood sugar and even the low points on the curves are above the initial sugar values.

Later, in spite of continued treatment, the "fasting" blood sugars remain low.

It is possible that higher values escaped our notice in the interval between December 11 and January 6, during which no analyses were made. But we can hardly attribute to chance the apparent relative constancy of the sugar values observed on November 30, December 9 and 11, and January 6, 9 and 11, in four of the five sheep then under observation. It was probable therefore that at that time, two to three months after the beginning of the injections of thyroxin, a condition of partial depletion of the glycogen store had been produced, and the glycogenolytic stimulus of the dose of thyroxin employed was inadequate to produce appreciable increase of blood sugar, even temporarily. Whether larger doses of thyroxin and thyroid extract would produce any changes was tested in experiment 2.

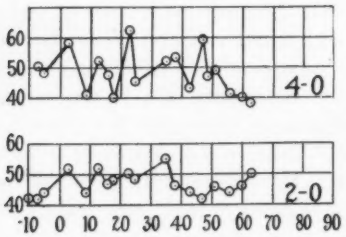

Fig. 3

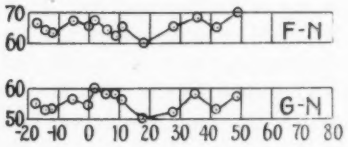

Fig. 4

Fig. 3. Cretin ewes. Effects of increase of thyroxin on 4-O, and of thyroid extract on 2-O.

Ordinates, milligrams of sugar in 100 cc.; abscissas, days after the change in treatment.

Fig. 4. Normal ewes. Controls in experiment 3.

Ordinates, milligrams of sugar in 100 cc.; abscissas, days after treatment began in experiment 3.

Experiment 2. The dose of thyroxin given to 4-O was increased on January 16 to 0.5 mgm., and that of thyroid extract given to 2-O was increased to 0.5 gram. The drugs were administered every other day until February 15. Determinations of blood sugar were continued until April 5.

The effect of the larger dose of thyroid extract was not very pronounced. The average blood sugar of 2-O was somewhat higher after January 16 than during the period just preceding, and about two weeks after the discontinuance of the treatment the average blood sugar level again declined. These may be taken as indications of a positive though slight effect.

In the case of 4-O, the cretin treated with an increased dose of thyroxin, the effect is much clearer. The blood sugar rose from 48 mgm. to 58 mgm. three days after the change of dosage. The higher value was not maintained, and the range of the fluctuations that followed (about 20 mgm., with a minimum of 40 mgm. and a maximum of 62 mgm.) was greater than had been observed in any untreated sheep—normal or cretin. The average blood sugar was not affected, remaining about 50 mgm. per 100 cc. as compared with values between 63 and 72 mgm. observed in the same sheep early in experiment 1. The difference seems to be due, in part at least, to the use of "fasting" blood in the later experiment, recently ingested food contributing to the higher sugar level in experiment 1.

It seems therefore that, under the conditions described, with the glycogen reserve partially depleted by previous treatment with thyroxin, an increased dose produces no change in the average "fasting" blood sugar, but disturbs its control, and causes wider fluctuations.

Experiment 3 was undertaken to test the effect of thyroxin upon the "fasting" blood sugar of normal sheep whose glycogen reserve had not been affected by previous thyroxin treatment.

Experiment 3. Preliminary tests were made between January 6 and 23. A slight downward trend is shown between January 6 (−17th day) and 11 (−12th day) by all but one of the sheep for which data are available for that period (F-N, G-N, H-N, I-N, 13-W, 16-W; 10-W is the exception). Five of the twelve sheep show definitely increased sugar values on January 18 (−5th day) as compared with January 11 (−12th day) (F-N, G-N, H-N, I-N, 18-W); 2 show indications of increases (7-W and 13-W); 4 show identical or practically identical values on the two days (2-W, 8-W, 12-W, 16-W); one is doubtful (10-W). A similar parallelism is apparent between January 18 (−5th day) and 23 (day of beginning injections). The sugar curves of the controls were parallel throughout the test, with minor exceptions.

These parallelisms had been observed in previous tests. It is obvious that there exists a common cause for these variations, to which all or almost all sheep respond alike. If that cause is in the diet of the sheep, there may be a means of so controlling it as to secure relatively constant blood sugars from day to day. The assumption of the dietary factor as a cause is strengthened by the observation that when diarrhea appears in the flock it generally affects all or nearly all normal sheep (the cretins rarely develop diarrhea).

Treatment of the lambs was begun January 23, 1923. Relatively large doses were employed, as follows:

F-N (76 pounds) and G-N (82 pounds) received no treatment.

5-W (62 pounds) and 12-W (64 pounds), ewe lambs, received 0.25 mgm. thyroxin, or about 0.004 mgm. per pound.

2-W (82 pounds) and 10-W (84 pounds), ewe lambs, received 0.5 mgm. thyroxin or about 0.006 mgm. per pound.

H-N (85 pounds) and I-N (79 pounds), ewe lambs, received 1 mgm. or about 0.012 mgm. per pound.

7-W (93 pounds) and 16-W (87 pounds), male lambs, castrated; and 13-W (82 pounds) and 18-W (85 pounds), normal male lambs, were injected 0.5 mgm. thyroxin each.

Subcutaneous injections were administered every other day from January 23 to February 15, inclusive.

It is apparent from the curves obtained (figs. 4, 5 and 6) that *the mean fasting blood sugar in normal sheep is higher under thyroxin treatment*

TABLE 2

Weights of treated sheep during the period of treatment

	JANUARY 18	FEBRUARY 16	GAIN
5-W	60	63	3
12-W	64	66	2
2-W	82	84	2
10-W	82	86	4
H-N	88	85	−3
I-N	83	80	−3
7-W	92	94	2
16-W	86	89	3
13-W	87	90	3
18-W	84	85	1

than in the preliminary tests. It can be due only in small measure if at all to the effect of recently ingested food, and must be attributed largely to the use of the glycogen reserve.

If the fluctuations about this higher mean sugar value could be minimized and conditions ascertained under which a fairly constant sugar value could be established, a better measure of the quantitative effect of thyroxin and of the proportionality, if any, between the dosage of thyroxin and the increase of blood sugar could be obtained. A more secure basis would also be available for the comparison of the "antagonistic effects" of thyroxin and insulin in the regulation of blood sugar. If the stabilization of the higher blood sugar in hyperthyroidism cannot be obtained the total effect will have to be gauged by more frequent determinations.

The higher values during the period of treatment were not due to more abundant diet. The weight records for the sheep under treatment show only slight gains (see table 2), their diet at this time being hardly more than a maintenance diet. That the failure to gain is not due to

administration of thyroxin, is shown by the weights of a group of sheep not treated with thyroxin (see table 3).

TABLE 3

Weights of untreated sheep during the same period

	JANUARY 18	FEBRUARY 16	GAIN
A-N	81	85	4
C-N	91	91	0
D-N	81	81	0
E-N	86	82	−4
F-N	76	78	2
G-N	82	80	−2

H-N and I-N, receiving the largest doses, showed the quickest response, and the effect continued longer. Of the other sheep the only ones which showed a pronounced rise during the first week were 10-W and 12-W.

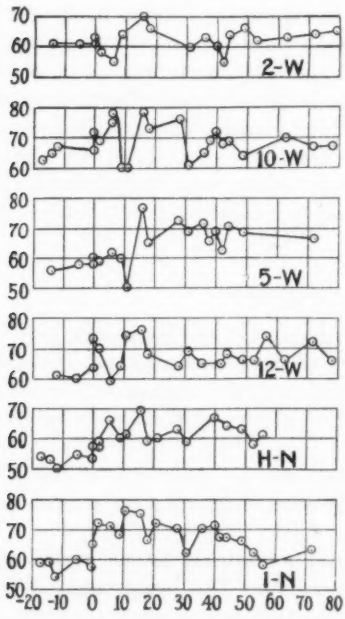

Fig. 5

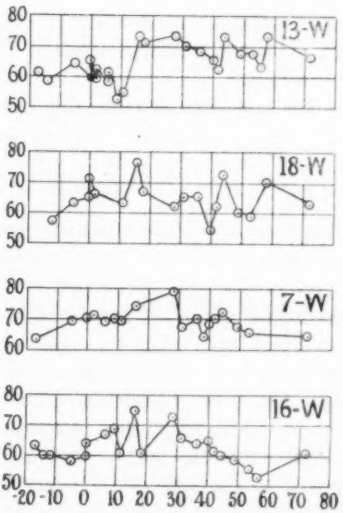

Fig. 6

Fig. 5. Normal ewes, treated with thyroxin.

Fig. 6. Male sheep, treated with thyroxin. 13-W and 18-W, normal; 7-W and 16-W, castrated.

Ordinates, milligrams of sugar in 100 cc.; abscissas, days after treatment began. Treatment was discontinued after the 23rd day.

10-W had previously been noticed as very excitable, submitted with difficulty to handling, and from the beginning showed somewhat higher blood sugar than is found normally.

No marked difference is shown between the effects of the dose of 0.004 mgm. per pound and 0.006 mgm., possibly because weight alone is not a satisfactory basis for dosage. It is possible, however, that more frequent analyses would have yielded a more accurate estimate of the total effect.

There may be seen in figure 6 a suggestion of more lasting effect of thyroxin treatment on the normal rams (13-W and 18-W), as compared with the castrated animals (7-W and 16-W). The comparisons at this time are, however, necessarily rough. When we have more accurate means of comparing the total effect of thyroxin on the blood sugar, we shall be able to test this point.

EFFECT OF SINGLE ADMINISTRATION OF THYROXIN. Some data were obtained incidentally which show that a single administration of thyroxin results in temporary increase of the blood sugar. The ordinary routine in the above experiments required that the blood be taken before the injection of thyroxin. In some cases another sample was taken at various intervals after the injection. The increases in blood sugar observed were sometimes considerable, in other cases small, but even the latter are considered significant in view of the consistency of these observations. Whenever two determinations of blood sugar were made—before and after the injection of thyroxin—the second value was invariably higher (0 day,[3] figs. 5 and 6). See footnote, p. 499.

SUMMARY AND CONCLUSIONS

Observations of the effect of thyroxin upon the blood sugar of sheep were made under conditions favoring the demonstration of alimentary hyperglycemia, and of the part of thyroxin in the regulation of "fasting" blood sugar when the glycogen reserve is low, and when the glycogen reserve is high.

Subcutaneous administration of thyroxin to sheep raised the blood sugar. Small but consistent increases were observed soon after a single injection. This effect was temporary.

Larger increases were observed after a variable latent period when thyroxin was administered every other day. The blood sugar did not stay at a uniformly high level, and wide fluctuations were observed.

In thyroidectomized sheep the average blood sugar was raised only during the first weeks of treatment when they were on adequate diet and when the blood sugar was taken after a morning in pasture; during this

[3] The day the first injection was administered.

period, the fasting blood sugar would probably have been raised by thyroxin, at the expense of the glycogen store, present at the beginning of the experiment.

The fasting blood sugar in the cretin sheep, whose glycogen reserve had been presumably partly depleted by three months' treatment, was about 50 mgm. per 100 cc., a value similar to that found in untreated cretins. Increased doses of thyroxin produced wide fluctuations of the blood sugar, the range being between 40 mgm.—a low value even for a cretin—and 60 mgm. The mean value for the blood sugar was not affected.

The mean "fasting" blood sugar of normal sheep was raised by thyroxin, presumably at the expense of the glycogen store.

The effect of thyroid extract and of sodium iodide on the blood sugar was not indicated clearly.

The course of the blood sugar curves was parallel. Nearly all the sheep showed peaks (or low points, as the case might be) on the same days. A common cause for the fluctuations is therefore indicated.

The fluctuations were within a small range in untreated animals, but within a wide range in those treated with thyroxin. The total effect of thyroxin on the blood sugar is therefore difficult to estimate accurately unless frequent determinations are made or a way found to stabilize the higher blood sugars nearer to their mean value. The latter might be feasible if the common cause for the fluctuations were identified. It is possibly dietary.

These experiments were aided by a grant from the Heckscher Research Council of Cornell University, made to Dr. Sutherland Simpson.

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STUDIES OF THE THYROID APPARATUS

XXVI. Correlation Between Thyroid Weight and Body Weight

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The restriction of a problem to a single method of investigation limits the adequacy of the generalized conceptions. It is therefore worth while to collect data and study them from more than one point of view.

In the previous papers of this series attention has been focused upon the relation between the thyroid gland and the processes of mammalian growth. The effect of experimentally induced thyroid deficiency on the rate of growth of the various organs of the body has been reported for two age series of albino rats of both sexes. Continuance of the investigation for other age series is in progress.

This paper is a report of a study of the relation, not between the thyroid and rate of growth, but between the thyroid size and body size as determined by weight values. It is less directly a study of the rôle of thyroid activity in growth, but is nevertheless closely correlated with the general problem as will appear directly.

The chief function of the thyroid is essentially that of a regulator of metabolism. It is probable that the rôle of the thyroid in growth is largely a matter of the exercise of this function. Now growth is ordinarily expressed by an increase in size of an organism. This increase in size is due either to increase in the number or the size or both of the constituent cells. This has been discussed in earlier papers (1), (2). While the two types of growth are interrelated in the growing multicellular organism, the relative participation of the two processes in the total growth changes with increasing age. The relative value of the growth by increase in cell number decreases with the age of the organism, while that of the growth by increase in cell size increases. Now increase in cell size is essentially the expression of a predominance of anabolism over catabolism. It is thus fundamentally a problem of metabolism, a process in which thyroid activity is an important participant.

From the foregoing it is evident that, other things being equal, there should be some correlation between body size as determined by weight at a given age and the level of thyroid activity. But we have no method of

directly measuring the grade of thyroid activity, nor if we had would it be feasible to determine this throughout the life course of a large number of individuals. Wide variations in thyroid activity are satisfactorily determined by basal metabolism measurements, but it is impossible at present to detect small differences in level which continuously present might well exert an effect upon the growth in weight or attainment of weight at a given age.

We can, however, start with the assumption that the weight of a presumably normally functioning thyroid in an animal of a given age is an index of its level of activity. Such an assumption is justified from the basal metabolic studies in physiological goiter.

It is necessary that the material for a study of this relationship be most carefully chosen if the data are to be validly useful for interpretation. It is necessary that in the groups to be examined the variability-producing factors of differences in age, sex, diet, environment, habitude, inheritance and disease be reduced to a minimum. This automatically rules out data from man. The ideal subject for a study of this type is the albino rat since with this animal maintenance of a high degree of constancy in the regulation of the objectionable variables is possible, and the number of individuals adequate for statistical analysis is easily obtained.

The data used in this paper were collected from 121 male and 121 female albino rats, 150 days of age. They were descendants of the experimental colony stock of "gentled" rats (3) and were thus of like inheritance, habitude and age. They were apparently free from infection, healthy and vigorous. The diet and environment was the same for all and as described by Greenman and Duhring (4). The two sexes were separated at weaning (23 days of age) and were never mated.

The body and thyroid gland weights of these animals were measured, and the correlation between them determined by the standard statistical method, an outline of which is well given in a recent book by Pearl (5).

In chart 1 are given the histograms of the frequency distribution of the body weights of the male and female rats. The general contour is that of a normal probability curve and needs no discussion. Chart 2 contains a like arrangement for the thyroid weights. Three facts appear. The scatter or variability of thyroid weight is much greater in the males than in the females. This is confirmed by the values for the standard deviations which are 0.0158 and 0.0067 respectively. The second is that the variability in thyroid weight is much greater than that in body weight in both sexes. This is confirmed by the values for the coefficients of variability which are for the thyroid: in the males 53.8, in the females 34.4, and for the body weight: in the males, 14.1, in the females 11.3.

The third point brought out in chart 2 is the high degree of asymmetry in the frequency distribution of the thyroid weights. It is similar in

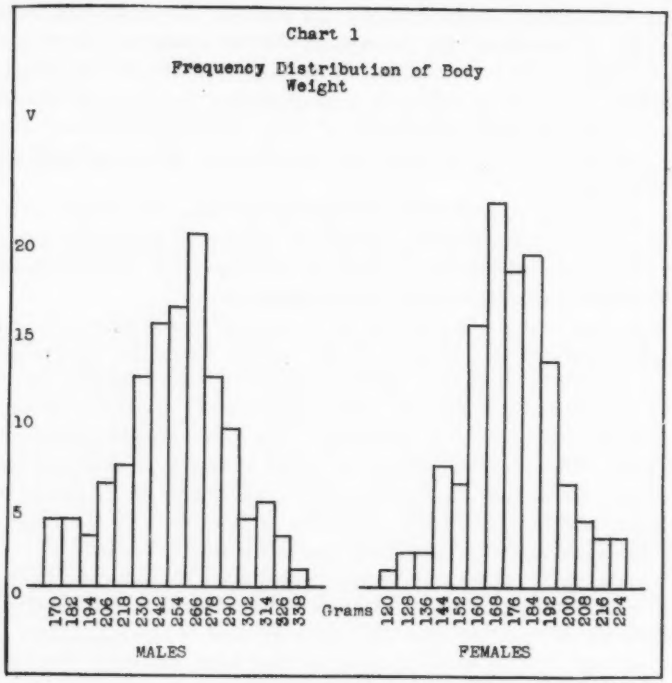


Chart 1
Frequency Distribution of Body Weight
V
20
15
10
5
0
170 182 194 206 218 230 242 254 266 278 290 302 314 326 338
Grams
120 128 136 144 152 160 168 176 184 192 200 208 216 224
MALES
FEMALES

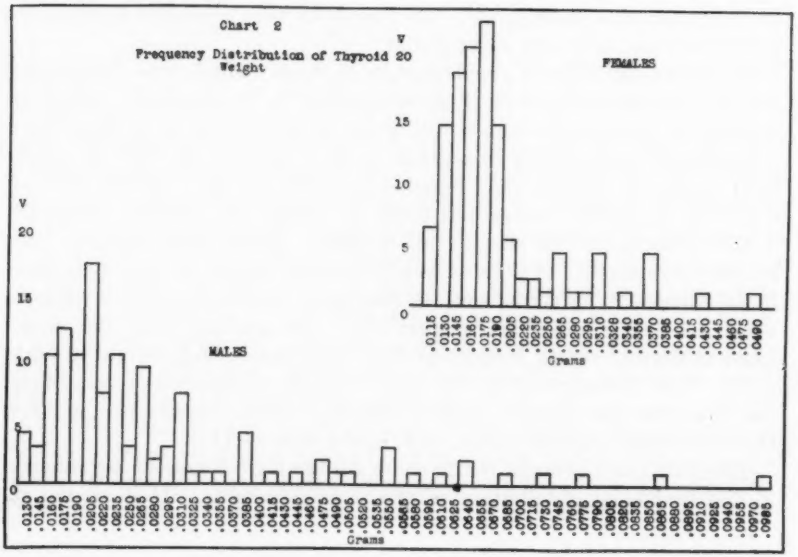


Chart 2
Frequency Distribution of Thyroid Weight
V
20
15
10
5
0
MALES
FEMALES
Grams

character in both sexes, and hence is significant and not fortuitous. Its causation is unknown. At first sight one might be inclined to reject from computation those values which fall outside the lower range where, by adequate choosing of the class, difference symmetry is exhibited. But such a procedure would be entirely unjustified since the character of the distribution is the same in both sexes, since a high percentage of the values fall beyond the range of symmetry, since the body weight distribution of the same groups is reasonably symmetrical, and since every effort has been made to reduce the variability-producing factors to a minimum and the stock was as uniform as is obtainable.

It should be noted that the symmetry is caused by the occurrence of thyroids of abnormally (?) heavy weight. This indicates that the sensitivity of the gland expresses itself rather in enlargement than in diminution of size. This sensitivity and the direction of its expression is also exhibited in man. It indicates a probably significant underlying similarity in functional purpose of the thyroid gland in the two species. It strengthens a bit the analogizing of findings in experimentation with the rat in this field, with clinical pictures of thyroid disturbance in man.

Turning now to the correlation tables 1 and 2 for males and females respectively it is seen that the distribution in each sex group taken as a whole fails to show a semblance of linearity or correlation. The coefficients of correlation for the whole number of variates, as given in table 3, show that apparently no correlation exists between thyroid weight and body weight. But when a line is drawn in the correlation tables through the class in which the mean thyroid weight falls, it is seen that there is a semblance of linearity to the right in the values below the mean in both sexes, and a hint of a trend to the left in the values above the mean. This indicates the possibility of two types of correlation between thyroid weight and body weight.

This possibility has been examined by a computation of coefficients of correlation of the two groupings in each sex. The one group was composed of the thyroid weight values below the mean and their corresponding body weight values; the other of the thyroid weight values above the mean and their corresponding body weight values. While I might have arbitrarily chosen a line of demarcation of the variates other than the mean of the total, and probably obtained coefficients of a higher order than those recorded, it seemed better to absolutely eliminate the possibility of personal bias and to take as the line of separation that value which denotes a legitimate statistical boundary, viz., the mean.

The coefficients of correlation for the several groupings are given in table 3, together with their probable errors and the number of variates. No question of spurious correlation can enter into these values because

there is great disparity in the absolute weights of the thyroid gland and the body as a whole. Separation of the sexes is necessary not only because

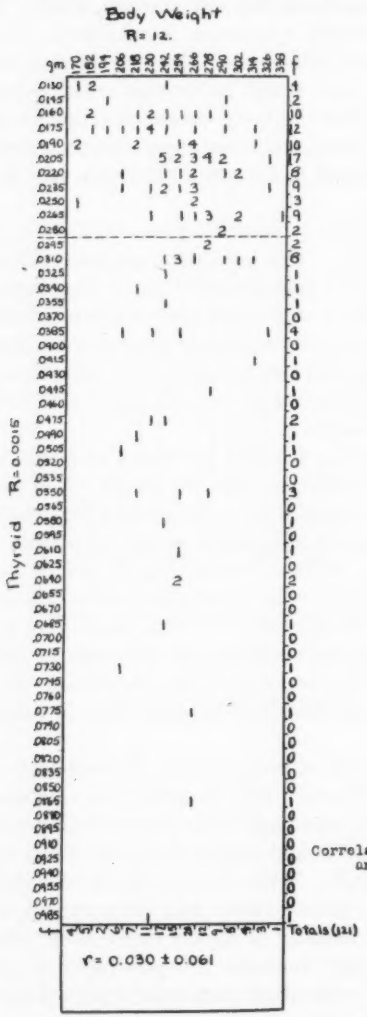
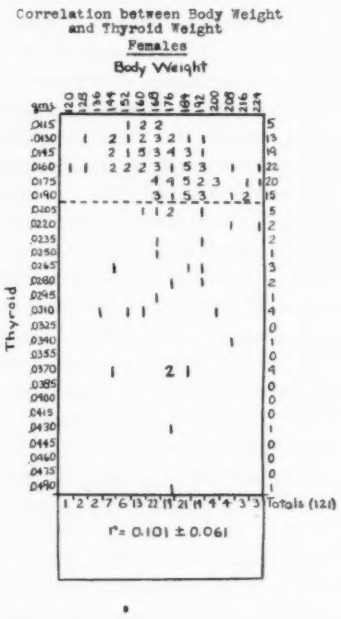


Table 1
Correlation between Body Weight and Thyroid Weight
Males

Table 2
Correlation between Body Weight and Thyroid Weight
Females



it is desired to see whether sex differences are present or not, but also because of the sex-difference in range and absolute values of the variables in rats of the same age.

The values in the first line of table 3 show definitely that there is no correlation between thyroid weight and body weight in either sex when all observations are compared.

To conclude from this that no correlation exists under any conditions would be wrong, as was pointed out in the discussion of the correlation tables, and shows that superficial acceptance of values without critical analysis may lead to fallacious interpretations. This is justified by the fact that the values in the second line of the table show that there is good positive correlation between thyroid weight and body weight when the thyroid weights below the mean of the total number are compared with their respective body weights. In the males the correlation coefficient is eight times the probable error, in the females it is six times. These magnitudes indicate certainty of correlation between the two variables.

TABLE 3

Correlation coefficients

	MALES		FEMALES	
	V	r	V	r
All values	121	0.030 ± 0.061	121	0.101 ± 0.061
Values below mean	86	0.427 ± 0.059	83	0.389 ± 0.063
Values above mean	35	-0.275 ± 0.105	38	-0.268 ± 0.101

This means that within this range a low thyroid weight tends to be associated with a low body weight and a high thyroid weight with a high body weight. It is noteworthy that the degree of correlation is practically the same in both sexes.

When the values above the mean, that is to say, the thyroid weights which are heavier than the mean of the total population, are compared with their respective body weights, there appears to be a negative correlation between the two as shown by the values in the last line of the table. Strict adherence to the statistical basis of interpretation would not allow a definite conclusion of this nature since the coefficients of correlation in both sexes are but two and a half times their probable errors. Nevertheless, I believe in this case that it is just to conclude the presence of negative correlation between heavier-than-the-mean thyroids and their body weights, because the direction of the deviation is the same in both sexes and opposite to that exhibited by the lighter-than-the-mean thyroids and their body weights, and because the degree of negative correlation is the same in both sexes; a sex-similarity which is also exhibited in the other group. It should be pointed out, however, that the degree of negative correlation in the one group is less than the degree of positive correlation in the other.

Granting that negative correlation exists under these conditions it means that within the above-the-mean range a heavy thyroid tends to be associated with a light body weight, and a light thyroid tends to be associated with a heavy body weight.

What is the significance of these relations? Are they interpretable on a basis of the present-day conception of the influence of thyroid activity on body weight?

Previous papers in this series have shown that thyroid deficiency causes marked retardation of growth in body weight. Hoskins (6), Schaefer (7) and others observed an apparent stimulation of growth in body weight on feeding small amounts of thyroid substance to rats. Carlson (8), Cameron and Sedziak (9) and others found that the feeding of larger amounts of thyroid preparations to rats caused them to lose weight. Loss of weight is a common accompaniment of hyperthyroidism in man. Thyroid medication is often used for purposes of weight reduction in adiposity. The nature of the influence of thyroid activity upon body weight is thus seen to be dependent upon its intensity. Up to a certain point increase in body weight (growth) is favored, anabolism is predominant. Beyond that point decrease in body weight (or prevention of normal increase) is caused, catabolism is preponderant or represents a greater proportion of the total metabolism.[1]

So in these weight relations;—up to a certain point thyroid weight and body weight increase side by side, beyond that point increase in thyroid weight is accompanied by decrease in body weight. The combination of these observations with those from the experimental side strengthens the assumption that (thyroid) organ size in normal individuals is an index of total organ effectiveness or activity. The correlations between thyroid weight and body weight fit in exactly with the deductions drawn from experimentation. They are particularly significant in that they were obtained from a group of animals living under uniform conditions and not subjected to the influences of thyroid feeding or extirpation.

The fact that body weight and thyroid weight are positively correlated does not of itself tell us whether the heavy body is heavy because the thyroid is heavy, or whether the heavy thyroid is heavy because the body is heavy. It is probable that inherited or congenitally acquired constitutional factors for greater growth of the body as a whole, entirely aside from thyroid function, are also conducive to greater growth of the thyroid as such. These are not to be neglected. But bearing in mind the extreme sensitiveness of the thyroid to stimulation with consequent response by increase in size and activity, and its intimate relation to growth it is more

[1] In conditions of thyroid deficiency retardation of growth is largely due to the lowering of the metabolic level rather than to a relative increase in catabolism.

probable that the heavier body is heavier because of the heavier and more efficient thyroid, than that the heavier thyroid is so because of the possession of a greater stimulus to its growth in common with the body as a whole.

Turning now to the region where body weight is negatively correlated with thyroid weight, a region where the intensity of thyroid activity is such that its effect upon metabolism is to increase catabolism relative to anabolism, we are justified in concluding from the combined evidence that the body of the lesser weight is so because of the thyroid of heavier weight, such thyroid presumably being above the average in total activity.

It is true that other organs than the thyroid participate in the determination of the body weight of a given individual. But the finger of experimental findings points directly to this organ as the dominant regulator of the metabolic processes on which rests the body weight of healthy, normally functioning individuals of uniform derivation and under uniform conditions of diet and environment. In this connection it should be noted that the degree of correlation in both brackets and the number of thyroids above-the-mean is practically the same in both sexes.

SUMMARY AND CONCLUSIONS

A statistical study of the correlation between thyroid weight and body weight of male and female albino rats 150 days of age is given.

It was found that body weight is negatively correlated with thyroid weight in both sexes when the thyroids above the mean value for the whole group are compared with their respective body weights.

When the thyroids below the mean value in weight of the whole group were compared with their respective body weights it was found that body weight is positively correlated with thyroid weight.

There is no sex-difference in the degree of positive and negative correlation.

An analysis of these findings is given in the text.

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THE OXIDATION OF CYSTINE AND CYSTEINE IN THE ANIMAL ORGANISM

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Evidence bearing on the existence and importance of cysteine in the animal body has been contributed by several investigators. The earliest of these were Baumann and Preusse (1), and Jaffé (2), who fed the monochlorine, monobromine and monoiodine derivatives of benzene to dogs, and isolated from the urine the corresponding mercapturic acids (acetylated cysteine conjugates of the benzene halides). At a later date Hefter (3) and Arnold (4) showed that nearly all tissues give a positive reaction with sodium nitroprusside and ammonia,—a sensitive and apparently specific test for the sulfhydril (−SH) group of cysteine; and Hopkins (5) actually isolated from tissues a dipeptide consisting of a molecule of cysteine apparently coupled with glutamic acid. Finally, Lewis and McGinty (6) recently fed phenyluramino cystine to rabbits and were able to prove that the substance was reduced, in part at least, to the corresponding cysteine compound. Not only does cysteine, like cystine, exist therefore in the living organism, but the one substance is perhaps just as important as the other; in fact, the conversion of the one into the other, and *vice versa*, may well be a normal metabolic reaction, and hence easily and exactly controlled by the living organism.

It is now generally agreed that in the natural process of metabolism, amino-acids in general are first deaminated either by a hydrolytic or oxidative reaction yielding a corresponding hydroxy or keto acid. The protecting of the amino group, therefore, by some inert radical should render the amino-acid impervious to the oxidative forces of the body. That this is true was first demonstrated by Magnus-Levy (7) who benzoylated and fed a number of the primary amino-acids. The same was later verified in our own laboratory (8) when we administered to animals a number of of phenylactylated amino-acids. Likewise Lewis (9) protected the amino groups of cystine with phenylisocyanate and found that the resulting uramino compound was much more difficult to oxidize than was cystine itself.

Cysteine, however, in addition to its α-amino group contains also a β-SH (sulfhydril) group, which may also be a sensitive point for oxidation.

In fact, it has even been suggested (10) that in the oxidation of cysteine the carboxyl (−COOH) group is the first that is attacked. Hence in the cysteine molecule there may possibly be at least three points at which the oxidative forces of the body can begin their work.

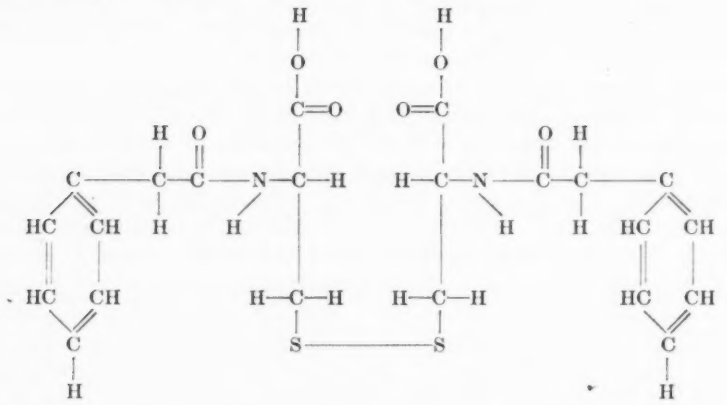
In an attempt, therefore, to ascertain the precise method of cystine and cysteine oxidation, and also to determine more accurately the relative importance in the organism of these two amino-acids, we administered a number of derivatives of cystine and cysteine which we had previously synthesized (11). In these derivatives the several sensitive groups ($-NH_2$, $-SH$, and $-COOH$) were blocked by some inactive radical usually aromatic in nature. Thus each of the amino groups of cystine was protected in one case with the phenylacetyl radical, and in another with the phenylisocyanate radical. Then the hydantoin of the latter compound was formed, thus blocking both the amino and the carboxyl groups simultaneously. In the derivatives of cysteine the sulfhydril group was blocked once with the benzyl radical, and at another time with the *p*-Cl benzyl radical. To protect the amino group alone we used phenylisocyanate, forming the phenyluramino compound. To cover both the sulfhydril and the amino groups simultaneously, we treated the benzyl cysteine in one case with phenylisocyanate, and in another with phenylacetyl chloride, forming respectively phenyluramino benzyl cysteine and phenylacetyl benzyl cysteine. Lastly, all three positions were blocked by converting the phenyluramino benzyl cysteine into the corresponding hydantoin.

There are certain cases in which the acetyl group is used by the animal body (12) for the detoxication of amino compounds. Would the acetylation of cysteine help or hinder its oxidation? If neither, what is the purpose of this means of detoxication? To clarify this point we used the acetylated benzyl cysteine and the acetylated *p*-bromophenyl cysteine (*p*-bromophenyl mercapturic acid).

These several compounds were administered to animals both *per os* and subcutaneously. This latter procedure obviated the possibility of a splitting in the gastro-intestinal tract either by acid hydrolysis or by bacterial action.

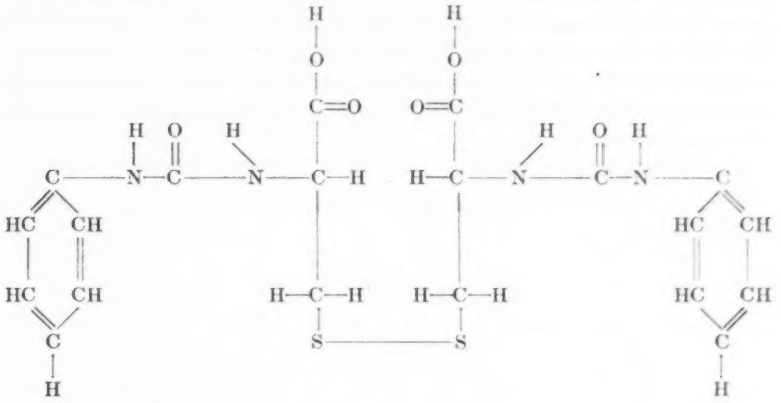
EXPERIMENTAL. Rabbits were used as experimental animals. Their diet was uniform throughout the experiment, consisting mainly of carrots. The substances were fed or injected, usually as water solutions of the sodium salts, made by dissolving the compound in dilute sodium hydroxide or sodium carbonate solutions. The animals were placed in small metabolism cages designed for the purpose, and the urine was collected in 24-hour periods. Total sulfur, inorganic sulfates and ethereal sulfates were determined on each urine collection, and from these figures the amount of oxidation of the substances was calculated. A criticism of the validity of this method will be found in the discussion.

1. Diphenylacetyl cystine



The amount of this compound fed was such that the rabbit received 320 mgm. of sulfur. It was found that 68 per cent of the sulfur was excreted in the form of sulfate sulfur, only 32 per cent of it appearing in the neutral sulfur fraction. After injecting 200 mgm. of sulfur in the form of the diphenylacetyl cystine, only 37 per cent of the sulfur was oxidized, the major portion (63 per cent) being found in the neutral or reduced fraction. About 8 grams of the substance were then fed in order to determine how much phenylacetyl cystine, if any, would be reduced to the corresponding cysteine compound. From the urine only 0.2 gram of the original diphenylacetyl cystine could be isolated. This was identified by its melting point. The presence of phenylacetyl cysteine was evidenced by the positive sodium nitroprusside test. We tried to isolate this substance, therefore, by extracting the evaporated urine with alcohol-ether mixture and taking up the residue in water, but we obtained only a gummy mass. This mass was then treated with benzyl chloride in order to convert any phenylacetyl cysteine into phenylacetyl benzyl cysteine. This, too, was unsuccessful. Hence we obtained only qualitative tests indicating the presence of a cysteine compound.

2. Phenyluramino cystine

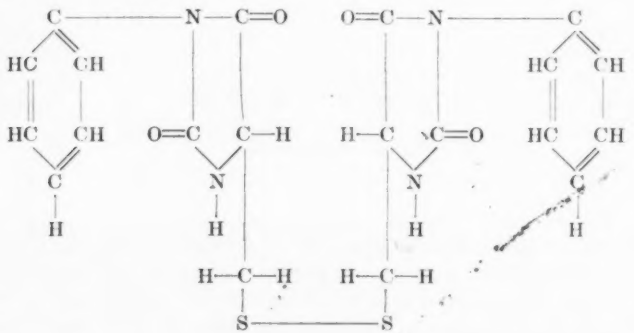


Sufficient of this compound was fed to furnish a total of 315 mgm. of sulfur. It was found that 41 per cent of the sulfur was excreted in the sulfate form, while 59 per cent was found in the neutral sulfur fraction. Two hundred milligrams of sulfur in the form of this same compound were injected subcutaneously, and of this amount only 13 per cent was oxidized to sulfates.

We again fed the substance to four rabbits until each had received 400 mgm. of sulfur. The united urines were acidified with acetic acid, extracted repeatedly with two volumes of ether, the ether solution immediately evaporated *in vacuo*, the yellowish, oily residue, which refused to crystallize, taken up in dilute sodium hydroxide, and shaken with a large excess of benzyl chloride for one hour. A substance was thus obtained which when purified and dried at 60°C. *in vacuo*, melted at 145–146°C.,—the melting point of phenyluramino benzyl cysteine. Analyses for nitrogen according to Kjeldahl showed 8.42 per cent and 8.47 per cent, instead of the theoretical value of 8.59 per cent. Hence, while unable to isolate the phenyluramino cysteine as such, we obtained this indirect evidence of its formation and excretion. Moreover, since this last reaction, i.e., benzylation, is known to be practically quantitative, we may well take it as a measure of the amount of phenyluramino cystine reduced by the body to phenyluramino cysteine and excreted before further oxidation. The amount of phenyluramino benzyl cysteine thus isolated was 1.65 gram, containing 162 mgm. of sulfur, or about 10 per cent of the total 1600 mgm. of sulfur fed to the four rabbits. This is approximately 17 per cent of the 59 per cent of this compound mentioned above as escaping oxidation in the organism of the rabbit.

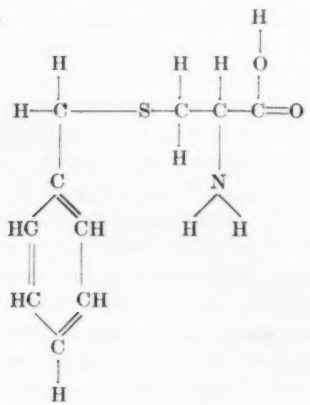
After the injection of 4 gram of phenyluramino cystine, i.e., 536 mgm. of sulfur, into two rabbits, we isolated from the urine 1.023 gram of phenyluramino cysteine by means of the benzyl chloride reaction. This signifies that 25 per cent of the sulfur fed was excreted as cysteine. We also recovered from the urine 1.205 gram of the original unreduced phenyluramino cystine, indicating that about 30 per cent of the substance passed through the organism unchanged.

3. Cystine phenylhydantoin.



Of this substance, 2.4 grams were fed, corresponding to 345 mgm. of sulfur. Of this, 41 per cent (141 mgm.) was oxidized to sulfates, while the remaining 59 per cent (204 mgm.) was excreted in the form of neutral or reduced sulfur.

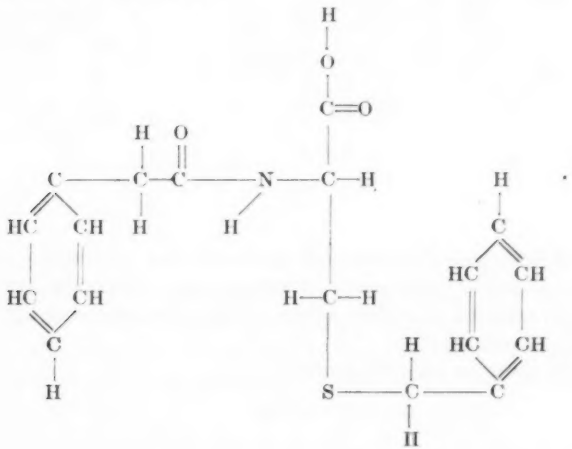
4. Benzyl cysteine.



One and two-tenths gram of this material was fed as a water solution of the sodium salt. This amount was equivalent to 183 mgm. of sulfur. Of this ingested sulfur, 58 per cent (106 mgm.) was oxidized to sulfates, while the remaining 42 per cent (77 mgm.) appeared as neutral sulfur.

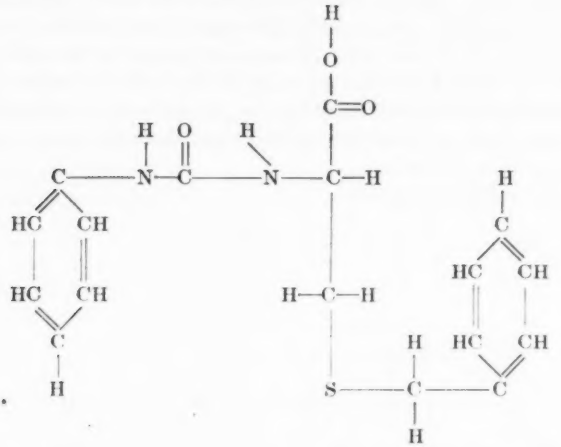
After the injection of 60 mgm. of sulfur in the form of benzyl cysteine, 22 mgm. were oxidized to sulfate sulfur, i.e., 36 per cent. Of these 22 mgm., 18 mgm. appeared as ethereal sulfates, and were therefore apparently oxidized to a benzyl sulfate.

5. Phenylacetyl benzyl cysteine.



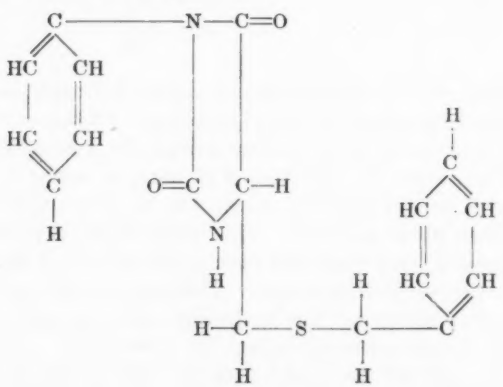
This compound was fed in such quantities that 117 mgm. of sulfur were given (1.2 gram of phenylacetyl benzyl cysteine). Of this sulfur, 37 mgm. (32 per cent) were excreted as sulfate sulfur, the remainder appearing as neutral sulfur. After the injection of 50 mgm. of sulfur in the form of the phenylacetyl benzyl cysteine, there was no perceptible rise in the amount of sulfate sulfur excreted. This would seem to indicate that in the gastro-intestinal tract there had been an hydrolysis of the compound, followed by absorption and oxidation. This reaction did not occur when the substance was injected. The hydrolytic cleavage probably effected the removal of the phenylacetyl radical, for it would be very difficult for the organism to split off the benzyl radical. This assumption is further borne out by the fact that benzyl cysteine, as shown above, is oxidizable in the body.

6. Phenyluramino benzyl cysteine.



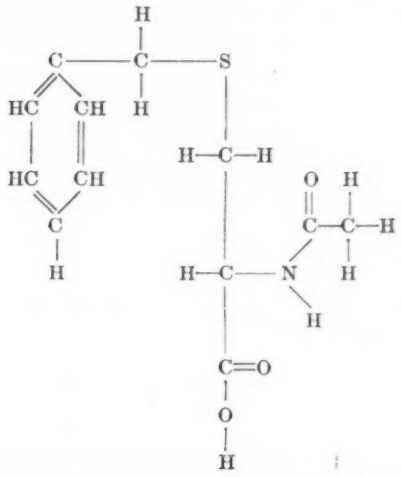
One and twenty-five-hundredths gram of this compound, containing 123 mgm. of sulfur, was administered *per os*. The substance did not increase the amount of sulfate sulfur in the urine, showing that the material was not oxidized.

7. Benzyl cysteine phenylhydantoin.



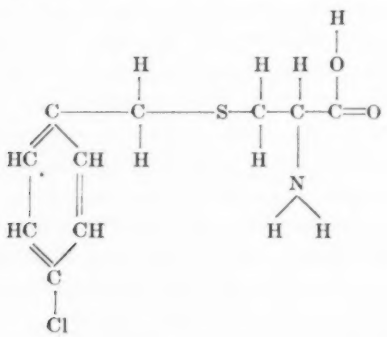
Of this substance, 1.25 gram was fed, containing 127 mgm. of sulfur. The substance apparently passed through the organism unattacked, for there was no increase in the amount of oxidized sulfur excreted during the experimental period.

8. Acetyl benzyl cysteine.



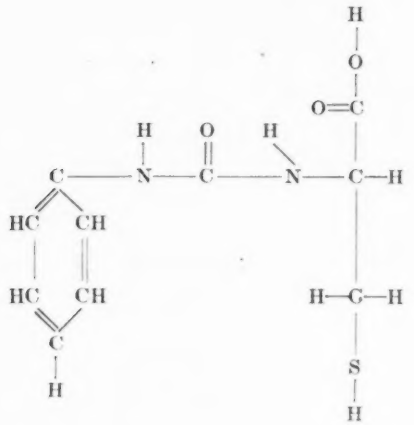
One and twenty-five-hundredths gram of this material was fed (153 mgm. of sulfur). The increase in sulfate sulfur following the feeding amounted to 89 mgm., i.e., 58 per cent of the sulfur fed. The percentage of sulfur oxidized, therefore, in this instance, was the same as in the case of benzyl cysteine itself.

9. p-Chlorobenzyl cysteine.



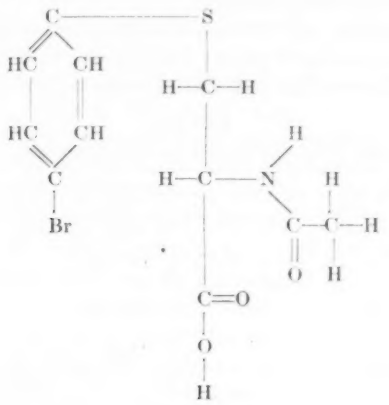
One and thirty-six-hundredths gram of this substance was fed, containing 186 mgm. of sulfur. Of this amount of sulfur, 98 mgm., or 53 per cent, were excreted in the form of oxidized sulfur.

10. Phenyluramino cysteine.



After feeding 2.42 grams of this substance, i.e., 321 mgm. of sulfur, 41 per cent of the sulfur, or 131 mgm., was oxidized to sulfate sulfur. This was almost exactly the same amount as was oxidized when phenyluramino cystine was fed. After injecting 200 mgm. of sulfur in the form of phenyluramino cysteine, we found that 48 mgm., or only 24 per cent of it, were oxidized to sulfate sulfur. Thereupon, 5 grams of the cysteine compound were fed, containing 666 mgm. of sulfur. The urine was collected, acidified with acetic acid, extracted rapidly with ethyl acetate to remove the unaltered phenyluramino cysteine, and later with acetone to extract whatever phenyluramino cystine might have been formed by the oxidation of the cysteine compound. The ethyl acetate extract was reëxtracted with ether several times. the ether evaporated in a current of warm air and the oily residue benzylated, converting the phenyluramino cysteine into its benzyl derivative. This was recrystallized, dried and weighed. The acetone solution was also evaporated to dryness and the residue twice recrystallized from hot water. Of the 666 mgm. of sulfur fed, 104 mgm. of it (14 per cent) were found in the form of phenyluramino cystine, and 186 mgm. (28 per cent) as phenyluramino cysteine.

11. p-Bromophenyl mercapturic acid.



This substance was fed in quantities sufficient to provide one rabbit with 200 mgm. of sulfur, and another with 300 mgm. of sulfur, i.e., 2 grams and 3 grams respectively of the substance. After feeding the 200 mgm. of sulfur, 30 per cent of it (60 mgm.) appeared in the urine as oxidized sulfur, and this in the ethereal sulfate fraction; and after feeding the 300 mgm. of sulfur, 69 mgm. of it (23 per cent) were oxidized to sulfate sulfur,—the ethereal sulfate fraction during that period registering this increase.

After feeding these different derivatives of cystine and cysteine, we finally fed free cystine and cysteine in quantities ranging from 0.8 gram to 0.95 gram containing 215 mgm. to 250 mgm. of sulfur. We found that 75 per cent to 90 per cent of both the cystine and the cysteine was each time oxidized to sulfates and so excreted.

Discussion. Evidently the protecting of the amino groups alone does not prevent the oxidation of the cystine molecule, for neither phenylacetyl cystine nor phenyluramino cystine escaped oxidation. Nor does the shielding of the carboxyl groups along with the amino groups modify the situation, for the feeding of cystine phenylhydantoin was followed by practically the same amount of sulfur oxidation as when phenyluramino itself was fed.

In the case of the cysteine derivatives, the blocking of neither the sulfhydril group alone nor of the amino group alone prevented the oxidation of the sulfur, as appears from the feeding of benzyl cysteine and phenyluramino cysteine. The protecting of both groups simultaneously, however, completely excluded oxidative operations, for phenyluramino benzyl cysteine passed through the organism unchanged. The same was

observed, of course, when all three positions were blocked, as in the case of benzyl cysteine phenylhydantoin.

Apparently, therefore, there are in the cystine as well as in the cysteine molecule, two very notable points of attack,—first and foremost, the amino group, and secondly, but still prominent, the sulfur group. The

TABLE 1

Sulfur from cystine and cysteine derivatives

SUBSTANCE	AMOUNT FED	AMOUNT INJECTED	AMOUNT OXIDIZED	
	mgm.	*mgm.*	*mgm.*	*per cent*
Cystine	215–250			75–90
Cysteine	215–250			75–90
Diphenylacetyl cystine	320		218	68
		200	74	37
Phenyluramino cystine	315		129	41
		200	26	13
Cystine phenylhydantoin	345		141	41
Benzyl cysteine	183		106	58
		60	22	36
p-Chlorobenzyl cysteine	186		98	53
Phenylacetyl benzyl cysteine	117		37	32
		50	0	0
Phenyluramino cysteine	321		131	41
		200	48	24
Phenyluramino benzyl cysteine	123		0	0
Benzyl cysteine phenylhydantoin	127		0	0
Acetyl benzyl cysteine	153		89	58
p-Bromophenyl mercapturic acid	200		60	30
	300		69	23

carboxyl group, however, does not appear to be a possible avenue for catabolic processes. That the sulfur group of cystine (−S−S−) is a vulnerable point is confirmed by the further fact that after the feeding of diphenylacetyl cystine the sodium nitroprusside test showed the presence in the urine of a cysteine compound, and that in the

case of phenyluramino cystine we actually isolated and identified, by means of benzylation, the cysteine compound. These last results give evidence that when cystine compounds are fed, the cystine is possible of conversion into cysteine. Moreover, the opposite reaction is a possible one, for after the injection of phenyluramino cysteine,—a cysteine derivative in which the amino group alone is protected—, we isolated from the urine and identified the corresponding cystine derivative, comprising 14 per cent of the original sulfur. Hence it would seem that the conversion of cystine into cysteine and *vice versa* may be a common metabolic reaction which is easily and exactly controlled by the living cell.

The covering of the amino group with an acetyl radical rather than with an aromatic one, apparently has no influence at all regarding oxidation, neither hindering nor facilitating it, for acetylated benzyl cysteine was oxidized in exactly the same proportion as was benzyl cysteine itself. Hence the acetylation of foreign organic compounds by the animal body, as occurs in the formation of the mercapturic acids and acetylamino benzoic acids, seems to have as its chief purpose the increasing of the solubility of the detoxication product with a simultaneous lowering of the surface tension of the medium (13).

It remains to justify our assumption upon which this experiment is based, namely, that the increase in the excretion of sulfates above that of the normal period is an index of cysteine (and cystine) catabolism. It has been objected that such an index was not had, on the ground that possibly the $-SH$ group may have been hydrolyzed, yielding on the one side a serine compound, and on the other an increase of inorganic sulfates (due to oxidation of the $-SH$). It is quite true that the $-SH$ is readily split off from the cysteine molecule by the hydrolytic action of dilute alkalis. But if such an hydrolysis had occurred in the present work we should have been able to identify the β-OH group of the resulting serine compound by means of the iron chloride test. Such tests, however, were always negative. Furthermore, if such an hydrolysis could occur when the sulfur group alone was blocked by some phenyl or benzyl radical, there is no reason for believing that it would not take place when the amino group also was protected. But then it would be quite impossible to explain the fact that when both the sulfur and the amino groups were protected no oxidation at all was observed. We must conclude, therefore, that the sulfur group is at least as stable as the amino group, and that the removal of the former by any of the body's metabolic forces is an indication of the catabolism of the original molecule.

SUMMARY

1. We prepared and administered to rabbits, both *per os* and subcutaneously, a number of cystine and cysteine derivatives in which either one, or two, or all three of the possible sensitive positions, i.e., sulfur group, amino group and carboxyl group, were protected by some aromatic radical.

2. Oxidation was not prevented either by the blocking of the amino group alone, or of the sulfur group alone, but only by the simultaneous blocking of both.

3. The blocking or non-blocking of the carboxyl group did not influence the oxidizability of the molecule.

4. There are in the cystine and cysteine molecules two sensitive positions, first and foremost, the amino group; secondly, the sulfur group. The carboxyl group is not a vulnerable point.

5. Apparently cystine is convertible into cysteine and *vice versa* by the organism of the rabbit.

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THE RELATIVE VOLUME OR WEIGHT OF CORPUSCLES AND PLASMA (OR SERUM) IN BLOOD

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Twenty-five years ago I showed how the fact discovered by me in 1896 (1), (2), that the conductivity of the erythrocytes is very small in comparison with that of the plasma or serum, could be applied to the determination of the relative volume occupied by the suspended bodies and the suspending medium (3). Two formulae were given, one of which fits slightly better with blood containing a high proportion of plasma and the other with blood containing a low proportion of plasma. In the middle range both gave the same results. The percentage error was shown to be small with either formula for any range. I have therefore habitually used the simpler of the two: $p=\frac{k}{k_1}(174-k)$, where p is the number of cubic centimeters of serum in 100 cc. of blood, k is the specific conductivity of the blood and k_1 the conductivity of the serum. The ratio $\frac{k}{k_1}$ is practically independent of the temperature, but as k appears also in the second factor of the right side of the equation the constant 174 is only correct for the temperature 5°C., at which the measurements were made or to which they were reduced. The values for k and k_1 are always the specific conductivities $\times 10^4$. This formula will be referred to as formula (*a*).

It may perhaps be worth while to state that in my studies of blood I have always employed bright platinum electrodes. These are better than electrodes coated with platinum black because they are so easily washed. As I make the measurements the minima are exceedingly sharp (4). In a recent paper a young investigator (5) has recommended the use of what he calls "blank" electrodes for some purposes for the reason mentioned. He is quite right, but he should not call them "blank." In this sense the word is German, not English. If it was ever employed in English with a somewhat analogous meaning, this use must now be considered distinctly obsolete.

This practical application of the fundamental fact described is far from being its most important consequence. But it affords, in my opinion, the most accurate method of measuring the relative volume of corpuscles

and plasma, and without any alteration whatever in the natural relations of the corpuscles (6). And as at last the matter seems to be arousing some interest even among clinicians, I have judged it worth while to point out, although it is sufficiently obvious, how the formula can be adapted to measurements made at any temperature. All that is necessary is to write it thus: $p = \frac{k}{k_1}\left(174 - k\frac{k\,(5°)}{k(t°),}\right)$, where k and k_1 are measured at temperature t°C., and k in the second member of the right hand side of the equation is multiplied by the ratio of k measured at 5°C. to k measured at t°C. This ratio can be determined once for all.

In figure 1 are plotted the values of k and k_1 for three specimens of blood (dog, calf and kitten). The specific conductivities $\times 10^4$ are plotted along the horizontal axis, temperatures along the vertical.

For the dog's blood $\frac{k\ (5°)}{k\,(18°)} = 0.717$, and the conductivities of the serum give the same ratio $\frac{k_1(5°)}{k_1(18°)} = 0.716$. $\frac{k\ (5°)}{k\ (25°)} = 0.616$; $\frac{k_1\,(5°)}{k_1\,(25°)} = 0.608$.

For the calf's blood $\frac{k\ (5°)}{k\ (18°)} = 0.718$, and $\frac{k_1\,(5°)}{k_1\,(18°)} = 0.714$; while $\frac{k\ (5°)}{k\ (25°)} = 0.620$, and $\frac{k_1\,(5°)}{k_1\,(25°)} = 0.613$.

For measurements made at, or reduced to 18°C. we can therefore write the formula $p = \frac{k}{k_1}\ (174 - 0.72\ k)$, and for measurements at 25°C., $p = \frac{k}{k_1}$ $(174 - 0.61\ k)$.

I have also employed another formula which can be used without adjustment for temperature, as it contains k and k_1 only in the form of a ratio: namely

$$\frac{k_1}{k} + \frac{1}{2} = \frac{174}{p} - \frac{3}{100 - p} \text{ (formula } (b))$$

The second member of the right side of the equation is introduced to give a closer approximation when p is large. When the proportion of serum is relatively small, say 50 to 60 per cent or less, the second member can for many purposes be omitted, the error thus introduced being less than 1 cc. of serum per 100 cc. of blood when $p = 50$, and growing continually smaller as p diminishes below 50. This range, as is seen from table 1, would include a considerable proportion of persons taken at random. The formula would then become $\frac{k_1}{k} + \frac{1}{2} = \frac{174}{p}$

The proportion of serum in the calf's blood by formula (a) was 61.2, by formula (b) 60.8 per cent. The hematocrit gave the following results: 33.5 (21 minutes), 38.5 (30 minutes), 44 (45 minutes), 46 (60 minutes),

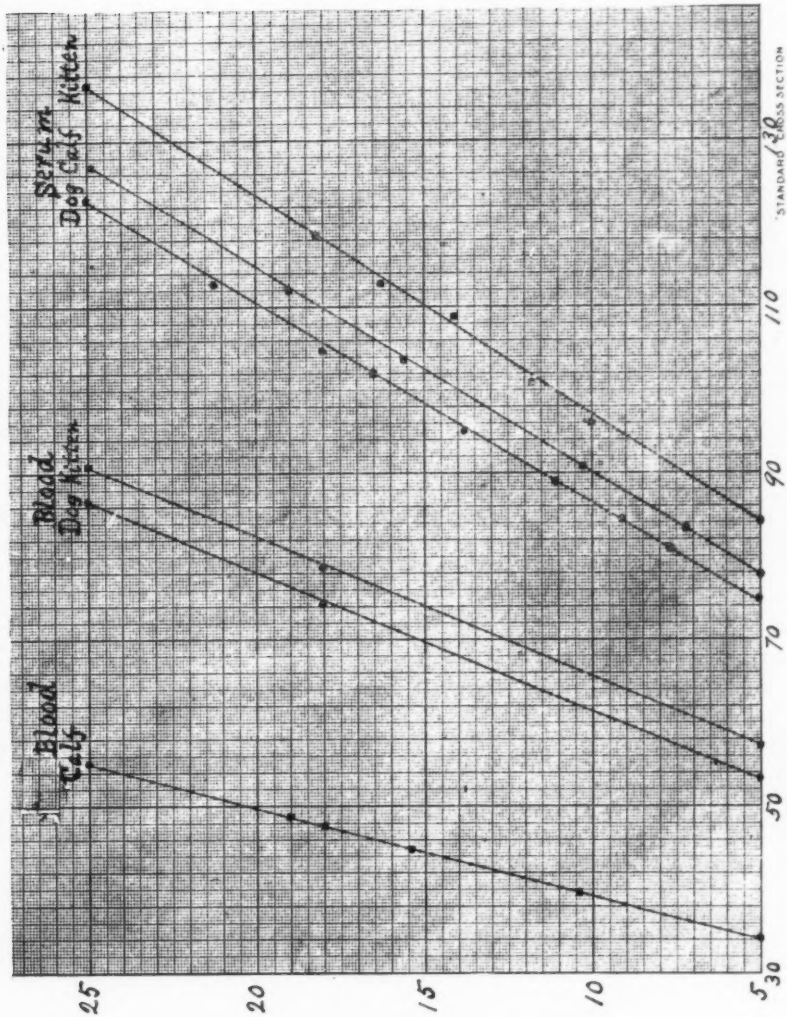
Serum
Dog Calf Kitten
Blood
Dog Kitten
Blood
Calf
STANDARD CROSS SECTION
30
50
70
90
110
5
10
15
20
25

Fig. 1

TABLE 1

	DATE	K × 10⁴ AT 5°C.		$\frac{k_1}{k}$	SERUM IN 100 CC. BLOOD		SOLIDS IN 100 GRAMS		RATIO BLOOD TO SERUM SOLIDS	
		Blood	Serum		Formula (a)	Formula (b)	Blood	Serum		
					cc.	*cc.*	*grams*	*grams*		
F. J.	v.17.22	32.8	76.5	2.333	60.5	59.9				
	v.18.22	35.0	79.8	2.280	61.0	60.9				After water deprivation
		34.4	77.7	2.259	61.8	61.3				After drinking
	v.24.22	34.0	77.7	2.285	61.2	60.8				
		35.6	79.0	2.219	62.3	62.2				
	v.29.22	32.2	77.7	2.413	58.8	58.3				After water deprivation
		36.0	80.3	2.231	61.9	61.9				After NaCl
	vi.1.22	35.1	81.6	2.325	59.7	60.0				After water deprivation
		35.9	81.6	2.273	60.7	61.1				After NaCl
	vi.5.22	34.6	81.4	2.353	59.2	59.4				After water deprivation
		35.3	81.4	2.327	60.1	60.0				After NaCl
E. M.	vi.5.22	28.4	79.0	2.782	52.3	52.1				Polycythemia
T.	xii.19.22	33.2	76.5	2.304	61.1	60.4				Normal. Before exercise
		33.3	77.2	2.318	60.7	60.2				After running 5 miles, much sweating
M.	iv.17.23	34.7	75.6	2.179	63.9	63.1	20.13			Before drinking
		34.9	74.9	2.146	64.8	63.8	19.68			Immediately after drinking
A.	iv.17.23	32.4	75.3	2.324	60.9	60.1	21.03			Before drinking
		26.4	71.0	2.689	54.9	53.5	22.33			15 minutes after drinking
Mrs. K.	v.12.23	29.3	76.3	2.604	55.6	54.9	22.42			Before drinking
		30.7	76.0	2.475	57.9	57.1	20.68			30 minutes after drinking
Mrs. Z.	v.12.23	24.7	74.4	3.012	49.5	48.7	24.94			Before drinking
		25.7	72.0	2.802	52.9	51.7	23.16			15 minutes after drinking

D. R.	v.14.23	35.3	72.9	2.065	67.1	65.6	17.64			Diabetes. Before insulin
	v.19.23	43.7	83.0	1.899	68.6	69.6	17.19	7.03	2.445	After insulin (urine sugar-free)
C.	v.19.23	29.8	77.0	2.584	55.8	55.2	21.91	8.98	2.440	Before drinking
		29.7	74.9	2.522	57.2	56.3	20.79	8.37	2.484	15 minutes after drinking
Ma.	v.19.23	33.0	77.5	2.348	60.0	59.6	20.77			Before drinking
		32.7	74.9	2.277	61.6	61.0	19.92			15 minutes after drinking
F.	v.26.23	32.8	80.6	2.457	57.5	57.5	20.98	8.34	2.515	Before drinking
		30.2	78.2	2.589	55.5	55.2	21.16	8.42	2.513	After drinking
Br.	v.31.23	30.6	76.3	2.493	57.5	58.1	19.83	9.54	2.078	Before drinking
		27.7	73.1	2.639	55.4	54.3	20.05	9.92	2.021	Immediately after drinking
Ga.	v.31.23	23.5	79.0	3.362	44.8	44.5	23.22	9.44	2.460	Before drinking
		18.8	73.7	3.920	39.6	39.0	24.59			5 minutes after drinking
G.	vi.9.23	29.9	80.0	2.676	53.9	53.7	21.29	9.51	2.237	Before drinking
		27.3	76.8	2.813	52.1	51.6	21.38	9.61	2.227	15 minutes after drinking
B.	vi.9.23	32.0	78.2	2.443	58.1	57.8	20.68	10.06	2.055	Before drinking
		30.7	75.8	2.469	58.0	57.3	19.64	9.68	2.029	30 minutes after drinking
S.	vi.16.23	26.0	78.0	3.000	49.3	48.9	23.20	8.99	2.581	Normal. Before exercise
		31.5	83.6	2.654	54.0	54.0	23.05			After exercise
R.	vi.16.23	28.8	78.7	2.733	53.1	52.9	22.14			Normal. Before exercise
		28.9	79.0	2.734	53.1	52.9	22.51	9.68	2.325	After exercise
W.	vi.16.23	28.6	80.8	2.825	51.5	51.3	22.71			Normal. Before exercise
		30.1	83.0	2.757	52.2	52.4	22.90			After exercise
L.	vi.16.23	30.8	79.0	2.565	55.8	55.6	21.70	9.75	2.225	Normal. Before exercise
		34.1	79.2	2.323	60.2	60.1	20.96	9.05	2.316	After exercise

49.5 (90 minutes), and 51 per cent (120 minutes rotation at about 3500 turns a minute). The blood sedimented slowly.

In the dog's blood the serum by formula (*a*) was 85.9, by formula (*b*) 84.8 per cent; by the hematocrit 67.5 (10 minutes), 78.5 (22 minutes), 79.5 (30 minutes), 80 (40 minutes), and 80 per cent (50 minutes).

For the kitten's blood formula (*a*) gave 79.6 per cent and formula (*b*) 81.8 per cent of serum. The hematocrit readings were 52 (5 minutes), 74 (15 minutes), 75.5 (25 minutes), and 76 per cent (35 minutes).

Table 1 contains the results of measurements on 14 patients and 5 healthy persons. The material was collected in a study of the water balance and water transportation in collaboration with Dr. C. D. Christie at Lakeside Hospital. We have published two papers on this subject (21), in which the conductivity of the serum and the relative proportion of serum and corpuscles were determined when water was long abstained from and then taken in large amount. The first work in which these methods were employed was by Wilson (22) under my direction. Wilson found that the drinking of water is followed by a small diminution in the specific conductivity of the blood serum, and this was long afterwards confirmed by Haldane and Priestley (23) and by Priestley (24), who, however, make no mention of Wilson's work. But the changes in our experience are slight, inconstant and often within the limits of the experimental errors. It should be noted that our observations were made on hospital patients, although not of course on persons who were markedly ill, while those of the other investigators mentioned were on normal persons.

Nearly all the observations in the table involved a comparison of blood specimens obtained after prolonged (voluntary) abstention from water with specimens obtained after drinking as much water as possible, or a comparison (in the 5 normal persons) of blood drawn before and immediately after prolonged muscular exercise without drinking. In one patient a number of observations were made in which blood secured after deprivation of water was compared with blood obtained when considerable quantities of sodium chloride were then administered by mouth. The total solids were determined in the blood samples from most of the patients and normal persons, and in a good many the total solids of the serum likewise. The table is introduced to show how closely the serum percentages calculated by the two formulae agree. As the material will probably be used elsewhere in a joint paper by Doctor Christie and myself it is unnecessary to cite clinical details here. It may be pointed out that except perhaps in 3 or 4 cases, including the case of diabetes under insulin (D. R.), none of the procedures studied caused any unequivocal effect upon the conductivity of the serum. The stability of the serum percentage and of the total solids, both of blood and serum, was also remarkable, particularly in the normal persons after strenuous muscular exercise. The ratio of

the total solids of the blood to the total solids of the serum also altered little, if at all, showing that the corpuscles were not accumulating or losing water in a different proportion from the serum, as might be expected since they remain in osmotic equilibrium.

In table 2 the serum percentages calculated from the two formulae are compared on a series of dilutions of dog's erythrocytes with known proportions of the same dog's serum (7). The agreement is excellent. As regards the first blood specimen in the table, in which the percentage dis-

TABLE 2

BLOOD K × 10^4 AT 5°C.	SERUM IN 100 CC. BLOOD		
	Actually present	Calculated from	
		Formula (a)	Formula (b)
	cc.	*cc.*	*cc.*
5.2	9.3	10.8	10.8
8.9	17.7	18.1	18.1
12.4	25.5	24.7	24.6
16.1	32.2	31.3	31.2
19.6	38.4	37.3	37.1
23.2	43.9	43.1	43.0
26.5	49.0	48.1	48.1
29.8	53.6	52.9	53.0
33.4	57.8	57.8	57.9
34.6	59.0	59.5	59.6
36.5	61.6	61.8	62.1
42.7	68.2	69.0	69.6
47.9	73.7	74.4	75.1
52.8	78.2	78.8	79.7
57.0	81.9	82.1	82.9
60.5	84.7	84.6	85.2
65.8	88.6	87.7	87.9

Serum K × 10^4 at 5°C ≐ 81.2.

Formula (a): $p = \frac{k}{k_1}(174 - k)$.

Formula (b): $\frac{k_1}{k} + \frac{1}{2} = \frac{174}{p} - \frac{3}{100 - p}$

crepancy is greatest, it must be noted that this was the sediment of erythrocytes, necessarily mixed with some serum, from which all the following blood dilutions were prepared. The amount of serum in the sediment was estimated colorimetrically, but with the small percentage present the error in the colorimetric estimation was of course considerable. An error of 1 cc. in the serum would be more than 10 per cent. In the first dilution, in making which the added serum was of course accurately measured, this error would be already reduced to little over 5 per cent, and would

grow rapidly less in succeeding dilutions. No importance must therefore be attached to the difference in the calculated and the "actual" values for the first specimen.

Figure 2 shows the curves obtained on plotting the percentages of serum as ordinates and the ratios of the specific conductivity of the serum to that of a series of suspensions of corpuscles in the serum as abscissae. The

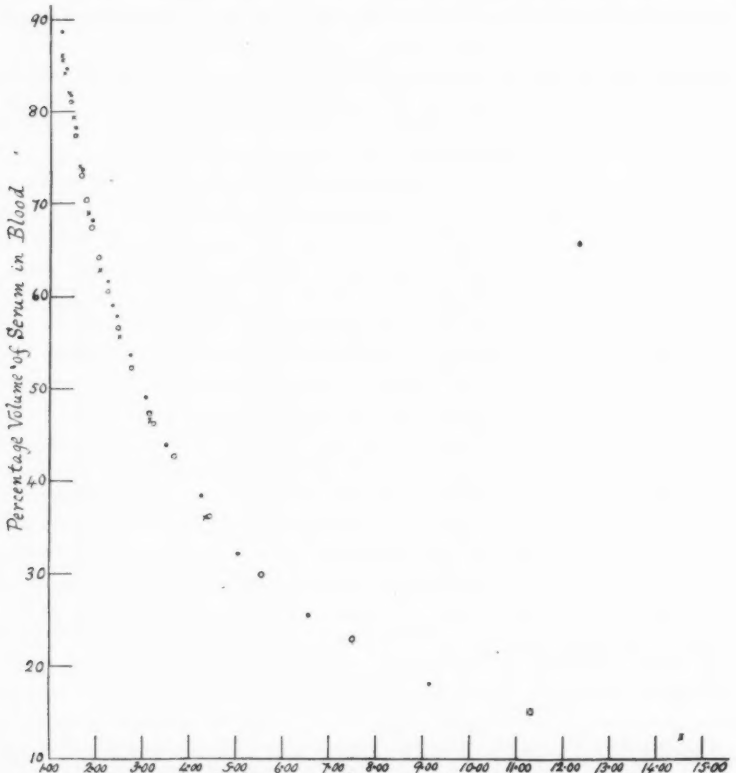

Fig. 2

results of three separate experiments with blood from different dogs are plotted. The points are indicated by dots (experiment of table 2), small circles and crosses respectively for the three experiments. It will be seen that the points belonging to the different experiments could be practically interchanged without altering the smooth curve appertaining to any one of them. The curve has not been actually drawn through the points to avoid

confusing the figure. The number of cubic centimeters of serum in 100 cc. of blood can be obtained by drawing a vertical line from the point on the abscissa axis representing the ratio of conductivity of serum to conductivity of blood and reading off the figure on the vertical axis corresponding to the point at which the ordinate cuts the curve.

Recently Fricke (8) has published an interesting paper, in which he treats mathematically the problem of deriving a formula whereby the specific conductivity of the cellular substance may be calculated from observed values of the specific conductivity of suspensions of the cells and of the suspending medium. His citation of the literature leaves something to be desired from the point of view of accuracy.

Thus he states that "Bugarszky and Tangl, Fraenckel, Oker-Blom, Roth, Stewart and others . . . . found that the red corpuscles act as perfect insulators, so that the electric current exclusively passes in the space between them." From the references given by him the reader is left to gather that my first paper on the subject was published in 1899, whereas those of Roth and of Bugarszky and Tangl were published in 1897. This is quite incorrect. The fundamental fact that the erythrocytes are practically non-conductors was first observed by me in 1896 in determining the output of the heart, by a method involving the measurement of the conductivity of numerous samples of blood collected before and after the injection of sodium chloride solution into the left ventricle. The fact was mentioned in a communication on the output of the heart presented to the American Physiological Society in December, 1896 (9). Details were published in the Journal of the Boston Society of Medical Sciences in June, 1897 (1). About a month later Roth (10) announced that the conductivity of the red corpuscles was so small that they could be considered non-conductors. I thereupon called attention to my results (2), and indicated how they could be used to determine the relative volume of plasma and corpuscles. A note by Bugarszky and Tangl (11) appeared almost at the same time, also stating that the erythrocytes were poor conductors and giving a formula for calculating their relative volume. The formula was based on the results of a very small number of experiments, and comparison was made merely with hematocrit determinations. The formula is therefore not at all accurate, as I showed later (3), and the authors state that in all probability they would have had to modify it if they had made a larger number of observations.

It is quite clear then that the relatively low conductivity of the erythrocytes was discovered independently by Roth, Bugarszky and Tangl and myself, although it happened that my work was published first. There is no basis for quoting Oker-Blom in this connection. His first paper (12) was published in 1900. It was to a great extent a repetition of my work on "the behavior of the hemoglobin and electrolytes of the colored corpuscles when blood is laked" (13), and contained tables and curves strikingly similar to mine, although no mention is made of any of my papers. Fraenckel (14), 7 or 8 years later, simply applied methods already worked out to the determination of the relative volume of corpuscles and plasma. A statement in a recent paper (15) that it has been shown by "Moore and Roaf and others that the specific conductivity for serum is several times that for corpuscles," and quoting in support of this a paper published by these authors in 1908, is partially correct, since it was "others" who showed it.

It is more important to note the general biological significance of the conductivity relations of cells and tissues, and this I pointed out from the beginning, showing, for instance, (1) how "the stability of the quantitative and even qualitative differences in the easily diffusible inorganic constituents, not only between blood corpuscles and plasma, but between the organized material and the liquid of the tissues in general" was involved in these relations. It was emphasized (1) that the facts under discussion had "an important bearing on the explanation of such phenomena as the difference of apparent resistance in the longitudinal and in the transverse direction in muscle and nerve, electrotonic currents, and other so-called polarisation phenomena, the high degree of polarisability of these tissues compared with the polarisability of the surface of contact of ordinary solutions of electrolytes, etc."

It was soon shown that leucocytes and other cells also had a relatively low conductivity, and facts were brought forward in favor of the assumption "that in general the cells of the animal body are bounded by an envelope relatively impermeable to the ions of the extra- and intracellular liquids" or to certain of them (13). It was remarked, however, that the low conductivity of the cells need not depend upon the existence of a histologically differentiated membrane nor even upon the existence of a physiologically differentiated "Plasmahaut" or envelope.

It was pointed out in connection with the action of saponin upon the conductivity of erythrocytes and leucocytes (16) that the increase of conductivity which is known to occur in dying muscle may be due not only to increased formation of ions in the muscular substance, but to a change of permeability of the muscular "membranes." "The fact that the resistance of muscle is much greater in the transverse than in the longitudinal direction suggests very strongly that the resistance of the sarcolemma, and possibly also of whatever envelopes enclose the sarcostyles, is greater than that of the conducting liquids between the fibres or between the fibrils. And if these membranes are relatively impermeable to the electrolytes during the life of the tissue, as they should be if the proper osmotic relations are to be preserved, it is quite probable that their permeability, and therefore their resistance, becomes altered when the tissue dies." In this connection the fact that perfectly fresh erythrocytes are less permeable to ammonium chloride than corpuscles which have stood for a few hours (17) is suggestive.

After death when bacterial growth takes place great changes in the conductivity of blood or tissues occur, owing to the decomposition of the protein molecules, etc., causing a great increase in the electrolytes as well as a diminution in the viscosity. The proteolysis and other changes, both in pure cultures of bacteria and in putrefying material like blood or

serum, can be excellently followed by freezing point and electrical conductivity measurements, as I showed long ago (18). Oker-Blom (25), Benson and Wells (26) and others afterwards used the same methods in studying the autolysis of blood, blood-serum, muscle juice, liver tissue, etc., with and without the presence of bacteria, and obtained similar results. But, while they state (26) that such methods have been seldom used for determination of the rate of disintegration of proteins, no reference to my work is made by these writers.

Finally, it may be permissible to recall in connection with the conductivity of erythrocytes, leucocytes and other easily suspended cells that a decided increase in the potential difference between the belly and the tendinous extremity of the frog's gastrocnemius was found to be produced by applying sapotoxin solution (in salt solution) to the muscle next the tendon (19), and to emphasize once more the importance for the study of the real mechanism of such phenomena of exploring the actions of such substances upon the physico-chemical properties of these relatively simple, isolated cells. It was pointed out that the electrical changes might be due either to a change in the permeability of the "membranes" or to the liberation of electrolytes in the fiber contents or to both. A similar though less marked increase is produced by sapotoxin in the current of rest of nerve. Seven years later, Höber (20) confirmed my result in an investigation of the effects of saponin and other substances on the resting current of muscle, but makes no mention of my work.

SUMMARY

Data are supplied for adjusting to any temperature at which the conductivity measurements are made the formula, $p = \frac{k}{k_1}(174-k)$, which relates p, the number of cubic centimeters of serum (or plasma) in 100 cc. of blood, k, the specific conductivity of the blood $\times 10^4$ and k_1, the specific conductivity of the serum $\times 10^4$. This formula was originally constructed for measurements made at, or reduced to 5°C.

Another formula is given which is practically independent of temperature since k and k_1 only enter as a ratio, namely,

$$\frac{k_1}{k} + \frac{1}{2} = \frac{174}{p} - \frac{3}{100-p}.$$

It is shown that the two formulae give results which agree well with the actual volumes of serum in artificially prepared suspensions of erythrocytes in serum. The results of applying the two formulae to the data from the bloods of patients and normal persons and of animals also agree closely.

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OPHTHALMIA AS A SYMPTOM OF DIETARY DEFICIENCY[1]

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The interest in the types of ophthalmia that are associated with errors of nutrition has been augmented by the growing recognition of the widespread appearance of such disease among mankind during and since the World War. The occurrence of xerophthalmia as a symptom of dietary deficiency has been stressed recently by Bloch (1); and Belgvad (2) has recorded no less than 434 cases of keratomalacia in children and 19 in adults during the years 1909–1920 in Denmark, where it was previously a rather infrequent disease. Clinicians believe that such ophthalmias may follow not only an actual shortage of vitamine A in the diet, but also failure to secure a sufficient absorption of this food factor owing to alimentary disorders.

Three years ago we (3) reported that our records of one thousand rats representing the entire group under study during one year, among which nearly one-half were on diets undoubtedly deficient in one way or another, failed to disclose a single case of eye disease in animals other than those on rations deficient in vitamine A. Among 136 rats on diets deficient in this food factor 69, or 50 per cent, exhibited the ophthalmias studied in our laboratory by Lambert and Yudkin (4). Although the percentage incidence of such eye disorder has varied in the reports from other colonies (5) there has latterly been a concordance of opinion that it represents a characteristic symptom of lack of vitamine A. The fact that not all rats placed on rations deficient in vitamine A exhibit readily detectable characteristic eye symptoms does not militate against the diagnostic significance of this ophthalmia. Occasionally animals die before their eyes are visibly affected. It may also happen that despite the shortage of vitamine A in the food intake the reserve of vitamine A in the body suffices to protect the animal for an unusually long period before detectable symptoms develop in the eye. This feature of the

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

temporary protective value of stored vitamine A has been stressed in particular by the Wisconsin School (6), which believes that the relative susceptibility of young rats to dietary shortage of vitamine A varies with the diet of the mothers during gestation and the period of lactation.

The possible body store of vitamine A thus may play a part in the early susceptibility to ophthalmia and must be taken into account in experiments designed to test for this vitamine. The necessity for carefully removing vitamine A from the food has long been recognized and various methods have been proposed for the preparation of diets as free as possible from this food factor (7).

To explain the fact that on a diet deficient in vitamine A not all animals develop ophthalmia Carlson (8) has suggested that a hereditary factor, predisposition or idiosyncrasy may account for the apparent immunity. In attempting to test this hypothesis, Hejinian (8) in Carlson's laboratory used a diversity of food mixtures, some of them apparently comparable to those which we have employed. The proteins ("meat residue" or casein) were reported to be purified so as to insure exclusion of vitamine A. Despite these precautions ophthalmia failed to appear. On the other hand "on a more natural and less purified diet xerophthalmia occurred in two out of three litters." The latter diet consisted of "steel cut oats 40 gm., gelatin (Bacto) 10 gm., dextrin 46.3 gm., salt mixture 3.7 gm." One thus gains the impression that a high degree of purification of the foods protects the animal rather than renders it more susceptible to a deficiency of vitamine A; or that some added factor in natural foods is requisite in conjunction with the lack of vitamine A for the genesis of this eye disease.

It happens that since our earlier report on ophthalmia and diet (1) we have conducted many additional feeding experiments on rats subjected to a deficiency of vitamine A in their rations. The purpose of the different tests was varied; among them was a considerable number in which the food ingredients, exclusive of the sources of vitamine B, were for special reasons submitted to an unusual degree of refinement. In general the experiments show that *with the use of the more highly purified diets the incidence of ophthalmia increased with the extent of purification of the food ingredients.*

The plan of the test usually involved feeding a mixture of protein—"meat residue," casein, lactalbumin, gliadin, or edestin—starch, a more or less "complete" salt mixture, vitamine B in the form of yeast or yeast fractions, and sometimes lard. Of approximately 4000 rats (nos. 6000–10376) the number of animals on diets containing little if any of vitamine A that were under observation 20 days or longer was 493. Among the latter *ophthalmia was actually recognized in 297 or 60 per cent.* This is even greater than the percentage incidence (50 per cent) in the earlier group of rats reported by us.

If we select the group living on diets for which the greatest degree of purification of the ingredients was undertaken the incidence of ophthalmia is decidedly larger: among 114 rats 94, or 82 per cent, showed unmistakable eye symptoms. Of these animals 88 were on diets in which edestin furnished the protein. The instance of ophthalmia among them was 83 per cent. Twenty-three rats on diets containing casein, purified by extraction with fat solvents, exhibited ophthalmia in 80 per cent of the individuals. Three rats on diets containing extracted "meat residue" all developed this disease. In some of these instances the salt mixtures were, for incidental reasons, "incomplete" with respect to the absolute quantities or relative proportions of calcium, magnesium or phosphorus supplied. McCollum, Simmonds and Becker (9) have reported that eye disease may arise as the result of unsatisfactory relations in the inorganic portions of the diet. That such a factor does not account for the higher incidence just noted is evident if we exclude all experiments with the "highly purified" diets in which any other than a demonstratedly adequate salt mixture (10) was employed. Among the remaining 64 rats 54, or 84 per cent, showed the eye symptoms. Stammers (11) has recorded an incidence of 88.6 per cent among young rats. The details of his food mixtures are not familiar to us.

In order that the reader may obtain some impression of what is meant by these "highly purified," "synthetic" rations attention is called to typical procedures adopted for the preparation of the foods in our last group just described. Thus one lot of rats was fed a mixture of edestin 16 per cent, starch 56 per cent, salt mixture 4 per cent, lard 24 per cent, along with 0.4 gram dried yeast daily. The protein, obtained by recrystallization, as well as the starch and the yeast were repeatedly extracted with hot alcohol and then with ether until neither removed significant quantities of soluble solids. In other tests casein purified by repeated reprecipitations and extracted in a similar manner with alcohol and ether replaced the edestin. Sometimes the Osborne-Wakeman yeast fraction which is practically free from everything soluble in alcohol or ether was used as a source of vitamine B in daily doses of 40 mgm. Another series of trials involved the use of "meat residue" and starch that were carefully extracted with ether. There were also groups that received no fat whatever in the diet. The diversity of conditions and the comparative "refinement" of the isolated food substances are thus emphasized.

Inasmuch as Hejinian's first failures to develop ophthalmia on "the so-called synthetic diet" involved the use of "meat residue" that has been a frequent source of protein in our experiments, we have collated the outcome of the feeding tests in which such material was used in our diets without vitamine A. Among 24 animals 20, or *83 per cent, developed ophthalmia:* and all of these were subsequently cured by administration

of some material containing vitamine A. Out of 107 cases recorded as ophthalmia in this paper, in which treatment with vitamine A was attempted, 100 or 93 per cent were cured.

Incidentally it may be noted that the "meat residue," as prepared and described by us (12), has proved to be a highly satisfactory source of protein for rats in literally hundreds of trials, where it furnished about 15 per cent of the calorific value of the rations fed. We are therefore at a loss to understand why the experience of the Chicago laboratory should develop "the fact that 'meat residue' may not be an adequate protein in the diet of the rat." Hejinian's product was obtained from commercial sources so that the mode of preparation is not known to us.

The anomalies presented by Hejinian's records are accentuated further by the peculiar behavior reported for rats on even the diets that might be expected to be "complete." Thus it is stated:

> In the first place, all the rats did not respond to the diet, when vitamin A was present, cod liver oil being substituted for part of the butter in some diets. Therefore substitution of aerated Crisco (cotton seed oil) as an "A-free" fat was not possible. In the second place, where response to the synthetic diet was made, loss in weight, rough hair coat and atonicity of the muscles occurred, but no eye symptoms. An unbalanced condition was present in a large number of the rats on an A-free diet. This condition was characterized by inability of the animals to walk or to hold a balanced standing position. Several of these animals tended to hold their heads to one side and showed a circus movement when walking. Thinking this condition might be related to polyneuritis, special care was taken to have more than the adequate amount of vitamin B in the diet. No change in symptoms occurred.

It seems possible that some of these animals were suffering from the "inner ear infection" that often prevails among rats (13). If so, this affection may be a manifestation of deficiency of vitamine A as are other instances of increased susceptibility to infection.

SUMMARY

The incidence of the characteristic ophthalmia in rats on diets deficient in vitamine A has been determined for a new series of experimental animals and found to be comparable with our earlier reports. This eye disease appeared more promptly and frequently among rats living on more highly purified foods than among those on the less thoroughly purified diets. This is contrary to the failures recently reported by Hejinian to produce ophthalmia in rats on "synthetic vitamine-A-free diets."

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THE EFFECT OF FREQUENT SAMPLING ON THE FORM OF BLOOD SUGAR CURVES[1]

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A previous paper (1) reported the observation that during intravenous injection of glucose in dogs, at the rate of 0.7 gram per kilo body weight per hour, the blood sugar concentration rose and remained elevated for the period of the injection. The injection usually lasted two and one-half hours, and the blood samples were taken not oftener than every ten minutes. In the course of these experiments it was observed that the level of the blood sugar concentration was subject to considerable fluctuation, such that the omission of one or two samples from a curve definitely altered the course of that portion. It was decided, therefore, to study the course of the sugar curve plotted from determinations made at intervals as short as possible.

METHOD. The procedures in general were those described in the earlier paper (1). Sugar and hemoglobin determinations were made upon blood samples taken from unanesthetized and later (exper. 3, 4) from anesthetized dogs during the intravenous injection of glucose in approximately 20 per cent solution, at the rate of 0.7 gram per kilo per hour. The unanesthetized animals had been trained for variable periods to lie without effort to rise, but were somewhat restrained by loosely fastening the feet and head to the table on which they lay. The glucose solution was delivered from a burette according to a schedule which indicated the position the meniscus should reach at the end of each minute. It was delivered in most of the experiments by a Woodyatt pump; in all, the attention of an assistant was given chiefly or wholly to the task of regulating the flow. It was ordinarily kept within a variation from the schedule of 0.2 cc., or 40 mgm. of sugar. This variation permitted an error of less than 4 mgm. per kilo and, therefore, approximately of less than 4 mgm. per 100 cc. of blood for dogs of the size used.

Blood was drawn from the external jugular vein. In most of the experiments it was preserved with potassium oxalate until measured; sugar was determined by the Myers and Bailey method (2) and hemo-

[1]The work herein reported was carried out during the incumbency of a Medical Fellowship assigned by the National Research Council, Washington, D. C.

globin by the method of Palmer (3). The blood samples were packed in a salt-ice mixture as soon as taken, and kept frozen until analysis could be begun. In the later experiments (nos. 3, 4) the samples were preserved with sodium fluoride and sugar was determined by the Shaffer-Hartmann method (4). Hemoglobin readings were made in the DuBoscq colorimeter, comparing solutions of 0.1 cc. of blood in 10.0 cc. of 1 per cent hydrochloric acid (5), using the first sample drawn as the standard. These changes expedited procedures, insured enough filtrate for three sugar determinations and avoided the necessity of having frequently to change color standards during a long series of sugar readings. The blood was diluted 1 in 13 (1 cc. blood, 9 cc. water, 1.5 cc. sodium tungstate solution and 1.5 cc. 2/3 N sulfuric acid). The filtration was carried out directly, without centrifuging.

TABLE 1

SAMPLE NUMBER	BLOOD SUGAR, MGM./100 CC.		
	Duplicates		Mean
1	181	187	184
2	188	183	185.5
3	182	186	184
4	187	182	184.5
5	186	187	186.5
6	186	189	187.5
7	188	182	185
8	183	182	182.5
9	185	191	188

Mean of the series of means	185.3
Standard deviation	±1.7
Limit of error (6 × S. D.)	10.1

In all sugar determinations 1 cc. quantities of blood were utilized. The blood was measured in 1 cc. Otswald pipettes, calibrated with mercury "to contain." Measurements of reagents were made from special burettes of small bore. As a check on the accuracy of the method under routine conditions the following consecutive determinations were made on a single sample of blood to which enough glucose was added to bring its concentration to the height ordinarily found at the fastigium of a sugar curve. (See table 1.)

In two experiments (nos. 3, 4) anesthesia was induced by the administration of isoamyl ethyl barbituric acid, hereafter referred to for the sake of brevity as "amytal."[2] It was described by Page (6) as having no disturbing effect upon the blood sugar level. Our observations in a number of experiments not here reported confirm his findings.

[2] I am indebted to Eli Lilly & Company for the courtesy of a supply of the drug.

Blood samples were taken at intervals of two to five minutes in the first two or three hours of injection, at longer intervals thereafter. Between thirty and fifty samples were usually used for each curve. These were analyzed in duplicate.

OBSERVATIONS. Five representative experiments from a series of ten are reported. In one of the five reported the glucose was injected in two periods separated by a short interval without injection. In the other four the injection was given during single periods lasting from eighty minutes to five hours. In two, the longest, anesthesia was used.

More detailed data of the experiments are presented in the protocols and figures. In figure 1 the values of the duplicate determinations are indicated by dots above and below the curves.

Experiment 1: In this experiment the exact height of the initial rise is unknown due to technical difficulties resulting in failure to obtain a sample between the twenty and thirty minute periods. The curve probably rose higher than shown. Definite oscillations appear that are well outside the limits of error of the method. The plateau portion of the curve is made quite irregular by the frequent oscillations, but evidently averages an almost perfectly horizontal course. This curve is an excellent example of what should be considered the average curve, in the light of the findings in other experiments not reported and in experiments with less frequent samples reported in a previous paper (1). It illustrates also how one experiment might give two curves differing rather markedly in their course if plotted from two different sets of infrequent samples, say every fifteen minutes. The hemoglobin percentage falls with the rise in sugar and remains about 10 per cent lower than the initial level. If hemoglobin changes are taken to be the results of dilution and concentration of the blood, and the sugar curve calculated to a constant blood volume, no essential change is made in the oscillations in the sugar curve in this or in other experiments.

Experiment 2: In the curve from this experiment several waves appear that are definitely beyond the limit of error. A variation is also seen in the fasting level. The hemoglobin percentages, not plotted, did not fall as the sugar rose, but maintained a practically constant level.

Experiment 3: Amytal anesthesia was used. The latter portion of the curve, covering two hours, is omitted from the figure. Several oscillations appear, and no tendency to return to the fasting level is shown throughout the period of five hours of injection. This curve obtained during anesthesia has no characteristics that would distinguish it from curves obtained without anesthesia. The hemoglobin percentage remained practically constant.

Experiment 4: Amytal anesthesia was used. The latter portion of the curve, covering two hours, is omitted from the figure for lack of space.

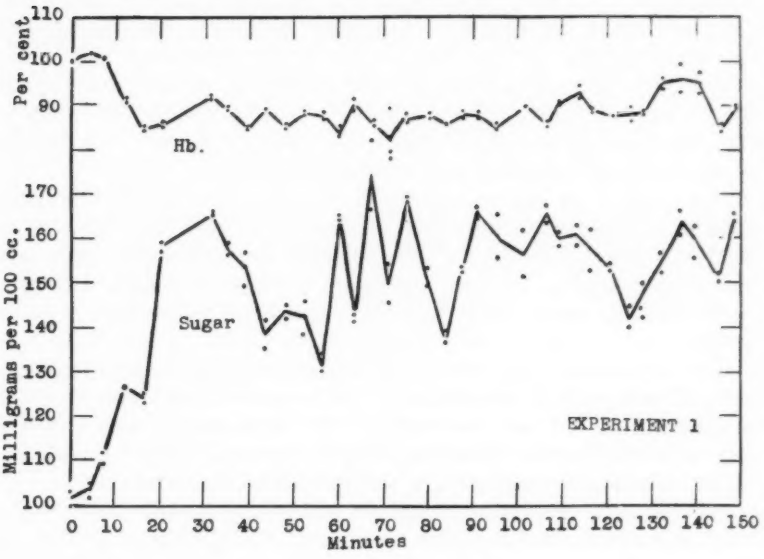
Per cent.
Hb.
Sugar
Milligrams per 100 cc.
EXPERIMENT 1
Minutes

Fig. 1

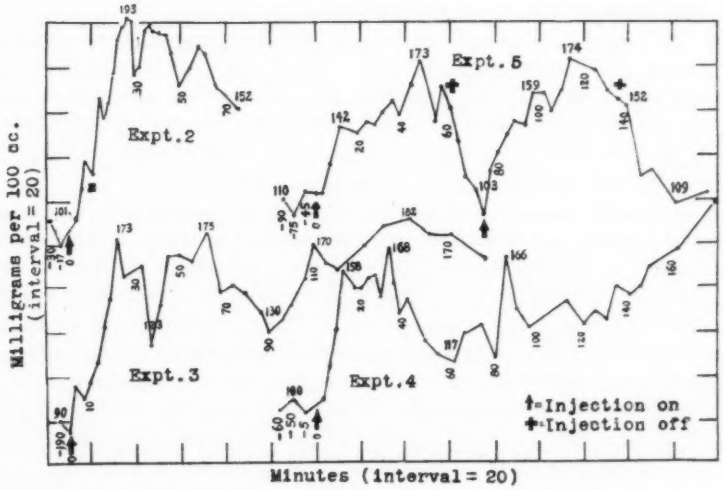
Expt. 2
Expt. 3
Expt. 4
Expt. 5
Milligrams per 100 cc.
(interval = 20)
†=Injection on
✚=Injection off
Minutes (interval = 20)

Fig. 2

The first three samples, taken before the injection began but during the anesthesia, show no significant variation. Several oscillations appear. This curve as well as that from the preceding experiment and others show larger undulations. No tendency to return to fasting level was seen throughout the five hours of injection. The blood sugar fell promptly when the injection was discontinued (not shown in figure). The hemoglobin percentage remained practically unchanged.

Experiment 5: In this experiment the injection was run for one hour, stopped for fifteen minutes, then continued for one hour more. The sugar level falls promptly when the injection is stopped but rises again when it is resumed. The two curves are the same in height, and very similar in their course, both gradually diminishing in the rate of their rise.

This experiment, with two other similar experiments not reported, makes it appear that hyperglycemia is an essential consequence of the injection as carried out, inasmuch as the blood sugar level, although lowered by stopping the injection, promptly rises again when it is resumed.

The foregoing observations may be summarized as follows:

1. Oscillations of varying amplitude and period usually occur in the blood sugar level during continuous intravenous injection of glucose, at the rate of 0.7 gram per kilo per hour. The amplitude averages about 15 mgm. per 100 cc. It is smaller when the general level of the curve is rising. The period averages about fifteen minutes. Comparable oscillations do not occur in the hemoglobin percentage.

2. The rate of rise of the blood sugar level on initiation of the injection is usually seen to gradually diminish. This is particularly evident in two experiments, not reported, in which the rise was unusually prolonged. In the experiments reported the slowing in the rise occurs only close to the apex and in some, if it occurs at all, it is so short as to escape detection by determinations made at the intervals used.

3. During the course of the injection for periods lasting up to five hours, the curve shows no tendency to return to fasting level. Discontinuing the injection, however, is promptly followed by a fall. Anesthesia with isoamyl ethyl barbituric acid, which apparently does not itself cause a change in level of blood sugar but certainly prevents any emotional reaction on the part of the animal, does not result in a lower curve or in its falling to fasting level during the course of the injection.

Discussion. The significance of the oscillations in the blood sugar curve is unknown, as, indeed, is true of physiological periodicity of many sorts. Their amplitude is ordinarily too great to be explained as experimental error. The absence of comparable changes in hemoglobin percentage makes it unlikely that these changes in sugar level are due to changes in concentration of blood solids in general. During the progress

of these experiments a paper by Hansen (7) reached our laboratory, reporting oscillations similar to those described herein. This investigator noted short oscillations in the blood sugar level of fasting patients, and of patients during alimentary glucose tolerance tests. The micro-method of Hagedorn and Jensen was used; 0.2 cc. of blood was drawn from the lobe of one ear. Samples were taken every five minutes. It was noted that synchronous oscillations occurred in the sugar level of blood from the lobes of both ears.

In experiments not here reported similar oscillations have been observed to occur during injection of sugar into mesenteric vessels.

SUMMARY

1. Blood sugar curves are presented, plotted from samples taken at intervals of from two to five minutes.

2. Frequent oscillations in the course of the blood sugar curves are described as characteristic.

3. The persistence of the elevation of the blood sugar level throughout the period of intravenous injection of glucose, as reported in a previous paper, is confirmed by experiments in which the period of injection was more prolonged, and in which an anesthetic (isoamyl ethyl barbituric acid) not affecting the blood sugar level was used.

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PROTOCOLS: *Experiment 1*: 11/9/23. Dog 36, M., tan collie, 13.7 kilos. Fasting 16 to 20 hours. Vigorous resistance on inserting needle, quiet thereafter. Thirty-six samples from the jugular vein in 155 minutes. Seventy-eight cubic centimeters of blood withdrawn; 108 cc. glucose injected; saline injected, 217 cc., of which 29 cc. given before glucose injection began. The glucose solution was 23 per cent.

Experiment 2: 12/2/23. Dog 40, M., tan, long hair, 16.6 kilos. Fasting 22 hours. No noteworthy evidence of emotion other than frequent attempts to scratch a mangy ear, which would cease when the ear was rubbed by the operator. Thirty samples taken in 140 minutes. Seventy cubic centimeters of blood withdrawn; 91.8 cc. glucose solution injected; 64 cc. saline injected before glucose injection began, 53 cc. more thereafter; total saline, 117 cc. Glucose solution was 23 per cent.

Experiment 3: 2/9/24. Dog 39, M., tan, long hair, 18.8 kilos. Fasting 41 hours when injection began. First blood at 6:50 a.m., immediately followed by administration of 0.85 gram amytal subcutaneously. Dog asleep by 10:00 a.m. and remained so throughout the day. At 180 minutes shivering. Temperature 37°C. Hot water bottles applied and shivering ceased. At 55 minutes injection stopped. Resumed at 60 minutes, 5 cc. behind schedule. Caught up 2 cc., but stopped again at 62 minutes. Began again at 66 minutes, 7 cc. behind. Stopped again at 86 and resumed at 88 minutes, 11 cc. behind. Remained behind schedule 10 cc., later 9 cc., until end of experiment. Forty-two samples taken. Forty of these taken in the period of

injection, 5 hours, 19 minutes. Eighty-four cubic centimeters of blood withdrawn; 342 cc. glucose solution injected (9 cc. less than schedule); 201 cc. saline injected.

Experiment 4: 2/12/23. Dog 43, M., Airedale, 22.25 kilos. Fasting 17 hours when injection began. At 5:25 a.m. 1 gram amytal (dissolved several days previously); 7:05 a.m., 0.2 gram. At 10:00 a.m., very drowsy, 0.2 gram amytal intravenously. From 11:00 a.m. to 12:00 m. dog "stretched" frequently, especially when touched. This ceased after 0.2 gram amytal intravenously at 12:15 m. Injection began at 10:25 a.m. Forty-five samples taken. Injection ran 4 hours, 20 minutes. Blood withdrawn, 90 cc.; glucose solution injected, 336.8 cc. of 1.2 cc. behind the schedule; saline, approximately 160 cc.

Experiment 5: 12/22/23. Dog 41, M., black, curly hair, 14.6 kilos. Fasting 15 hours. Trained two weeks, but continued very timid. Had a chronic cough. Coughed for 5 minutes, 20 minutes before injection began. At 118 minutes difficulty in reinserting jugular needle, with vigorous resistance by animal, otherwise no remarkable sign of emotion. Forty samples of blood taken in 265 minutes. Eighty cubic centimeters of blood withdrawn; 44.3 cc. of glucose solution injected in the first period, 44.7 in the second; approximately 75 cc. of saline given before the glucose injection began, 148 cc. more up to the end of the glucose injection, and 80 cc. after the end of the glucose injection; total saline, 303 cc.

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THE SUGAR OF ARTERIAL AND VENOUS BLOOD DURING THE ACTION OF INSULIN

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It has been found that the addition of insulin to Locke's solution perfused through the isolated heart of the cat or rabbit increases the rate at which sugar disappears from this solution (1). This led to the question whether a similar action of insulin could be demonstrated when the isolated hind limbs of a rabbit or cat were perfused at body temperature either with defibrinated blood or Locke's solution containing oxygen. In attempting such experiments care was taken to prevent any appreciable fall in temperature, or to allow the circulation to be interrupted prior to the start of the perfusion in the muscles. This was accomplished by inserting a cannula into the central end of one iliac artery, after ligating all branches going to the pelvis, and starting the perfusion through this cannula before cutting across the spine and removing the hind limbs to a large moist chamber, kept at body temperature. The fluid was pumped into the cannula under suitable pressure, and after its escape from the vein it was thoroughly oxygenated and again perfused. In most of the experiments the perfusion fluid consisted of defibrinated blood from the same animal, diluted with Locke's solution. It was found that the perfusion usually proceeded satisfactorily for some time (about thirty minutes), but that the muscles then rapidly became edematous, the perfusion fluid markedly reduced in volume and that the pressure necessary for perfusion also rose considerably. This made it impossible to use the method for the purpose of determining the influence of insulin on the rate at which sugar was being retained by the tissues, by comparison of the percentage of this substance in the arterial and venous blood.

The plan of investigation was therefore changed to one in which the percentages of sugar in blood removed simultaneously from the femoral artery and vein of an etherized animal were compared. It was realized that this method would be capable of revealing increased sugar retention by the muscles, as a result of the influence of insulin, provided only that this increased retention was very marked, because the great volume of blood flowing through the muscle in a unit of time would make it possible for a considerable change in sugar retention to occur without any measura-

ble change in the difference which normally exists in the percentages of sugar in the arterial and venous blood. By making the measurements at frequent intervals, however, it was considered possible that increased loss of sugar from the blood due to insulin might be demonstrated. In many of these experiments blood was also collected simultaneously from the vena cava opposite the liver, and from a branch of the portal vein, to see whether, by comparison of the sugar percentages, evidence of increased formation of glycogen in the liver, as a result of insulin, could be obtained. The technique followed was the same as that described elsewhere by one of us and R. G. Pearce (2) and M. E. Fulk (3). Long

TABLE 1

EXPERIMENT NUMBER	SUGAR PER 100 CC. BLOOD				
	Femoral artery	Femoral vein	Difference F.A.-F.V.	Portal vein	Difference F.A.-P.V.
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
3	302	302	0*	293	9
4	114			106	8
5	94	83	11	94	0
7	143	123	20	131	12
8	142	136	6		
10	121	107	14		
12	161	142	19	126	35
15	340	283	57*		
17	135	121	14	146	−11
18	173	147	26	162	11
19	174	158	16	174	0
20	232	224	8*	216	16
21	195	180	15	188	7
22	207	179	28*	194	13
23	221	187	34*	214	7
Average			19 or 16 omitting starred figures		9

glass cannulae were inserted in the *central* ends of the femoral artery and vein on one side, being pushed up so that the open ends lay in the iliac vessels close to the abdominal aorta and vena cava. In most of the experiments, ligatures were tied round the cannulae near their open ends. The blood collected through the cannulae, therefore, was that circulating through the vessels of the opposite limb. Similar cannulae were also inserted into the pancreatico duodenal vein (for collection of blood from the portal vein) and into the central end of the renal vein (for blood from the hepatic veins).

RESULTS. Many of the experiments were inconclusive because of insurmountable difficulties in securing samples of blood from all the vessels at approximately the same time. Since it was particularly with the collection of blood from the vena cava that most difficulty was encountered, blood from this source was omitted in many of the experiments.

The normal differences. An estimate of the differences to be expected without insulin will be gained by examination of table 1 in which the blood sugar percentages are given in the femoral artery and vein, and in the portal vein of a series of dogs, immediately after the cannulae had been inserted. In some cases, e.g., nos. 3, 15 and 20, a decided degree of ether hyperglycemia was present. It will be observed that the highest percentage of sugar occurred in the blood of the femoral artery in all save one case (no. 17) in which it was somewhat higher in the portal vein. This exceptional result was, however, only transient and in samples of blood removed twenty minutes later the highest percentage was found in the blood of the femoral artery. In nine cases the percentages in the blood of the femoral artery were above those in the portal vein, equal to it in two and lower in one. This shows that active hepatic glycogenolysis was occurring. More striking differences are apparent between the bloods of the femoral artery and vein, the former being decidedly above the latter in thirteen out of the fourteen cases and equal in the remaining one. The average difference between the bloods of femoral artery and vein was 0.019 per cent and that between femoral artery and portal vein 0.009 per cent. Omitting the values obtained when hyperglycemia was present (over 0.200 per cent blood sugar) the average difference is 0.016 per cent. These differences are somewhat greater than those observed by Pearce and Macleod and by Fulk and Macleod, in which the picric acid method was used for measuring the blood sugar, but, taking the results as a whole, they indicate that the liver, in ether anesthesia, is usually discharging glucose into the systemic blood, and that retention of glucose is occurring in the muscles of the hind limbs. The differences are greater than those obtained by Henriques and Ege (4) who, using Bang's method, made the observation on deglycogenated animals, and Cori, Cori and Glotz (6) who made the observations on partially starved rabbits, using the Hagedorn method.

The effects of insulin. The results of typical experiments in which insulin was injected into an etherized animal are shown in table 2, from which it will be observed that the relative sugar values did not become significantly changed by the injection of insulin. It should be noted that although the injection of insulin had no effect on arterial blood pressure, this fell later in three out of four of the experiments.

With the object of exaggerating the differences in blood sugar, if such would exist, the plan of the experiments was altered in that insulin was

given during the hyperglycemia caused either by injections of glucose or by epinephrin or asphyxia. As a preliminary to this it was necessary to study the levels when epinephrin alone was injected. Typical results are shown in no. 21 of table 3 from which it will be noted that the differences in the percentages in the various vessels were well within the normal range except for the samples removed in thirty minutes after injection (subcutaneously) when the portal vein showed the highest value. The low glycogen content of the liver in this animal is remarkable in view of the striking degree of hyperglycemia that occurred. No. 22 shows the results of an experiment in which a moderate dose of insulin was injected at the same time as epinephrin. The hyperglycemia persisted for a longer period than in the control experiment with epinephrin alone,

TABLE 2

EXPERIMENT NUMBER	SUGAR PER 100 CC. BLOOD					REMARKS
	Femoral artery	Femoral vein	Difference F.A.–F.V.	Portal vein	Difference F.A.–P.V.	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
6	94	83	11	94	0	Before insulin
	80	70	10	74	6	10 minutes after insulin
	70	55	15	58	12	*45 minutes after insulin
19	179	159	20	162	17	Before insulin
	150	129	21	134	16	35 minutes after insulin
	145	140	5	107	38	55 minutes after insulin
18	162	148	14	160	2	Before insulin
	138	126	12	140	+2	36 minutes after insulin
	135	107	28	117	18	48 minutes after insulin
	155	138	17	145	10	*100 minutes after insulin
4	103	96	7	99	4	Before insulin
	91	88	3	78	13	During injection
	61	83	+	74	+	*18 minutes after injection

*Arterial blood pressure very low (30 mm. Hg).

possibly because there was much more glycogen in the liver, but the relative values of the blood sugars do not deviate from the normal until somewhat over one hour after the injection, when there is evidence of rapid hepatic glycogenolysis.

In no. 20 the insulin was given one hour after the epinephrin and it caused a rapid fall in blood sugar without any significant alteration in the relationship of the percentages. We have consistently observed in all experiments in which the arterial blood pressure fell to about 60 mm. or less that the differences between the blood sugars became very much exaggerated. Results obtained under these conditions are therefore not

considered in this report. All of these experiments have been repeated several times with very similar results. The experiments in which glucose was injected were also unsatisfactory.

On account of our inability to demonstrate that insulin causes any significant deviation from the usual difference in the percentages of sugar

TABLE 3

EXPERIMENT NUMBER	SUGAR PER 100 CC. BLOOD					REMARKS
	Femoral artery	Femoral vein	Difference F.A.–F.V.	Portal vein	Difference F.A.–P.V.	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
21	195	180	15	188	7	After ether and operations
	370	340	30	389	±19	30 minutes after adrenalin
	397	383	14	410	+13	50 minutes after adrenalin
	325	316	9	319	6	70 minutes after adrenalin
	303	307	+4	317	+14	90 minutes after adrenalin
	280	269	11	275	5	110 minutes after adrenalin
	242	223	19	238	4	130 minutes after adrenalin
						Glycogen 0.09 per cent
22	207	179	28	194	13	After ether and operations
	212	187	25	204	8	25 minutes later
	241	224	17	235	6	12 minutes after adrenalin and insulin
	299	269	30	274	15	20 minutes after adrenalin and insulin
	315	299	16			30 minutes after adrenalin and insulin
	322	303	19	314	8	60 minutes after adrenalin and insulin
	384	349	35	331	53	90 minutes after adrenalin and insulin
						Glycogen 4.3 per cent
20	232	224	8	216	16	After ether and operations
	232	219	13	228	4	10 minutes later
	264	244	20	271	+7	10 minutes after adrenalin
	318	282	26	298	20	30 minutes after adrenalin
	292	286	6	291	1	60 minutes after adrenalin
	309	288	21	293	16	12 minutes after insulin
	285	260	25	266	19	30 minutes after insulin
	213	195	18	208	5	50 minutes after insulin
	213	199	14	208	5	60 minutes after insulin
	170	166	4	174	+4	90 minutes after insulin
	168	157	11	140	28	120 minutes after insulin
						(B. P. = 35 minutes Hg)
						Glycogen 0.36 per cent

in the arterial and venous blood and in that of the portal vein in etherized normal animals, we considered it advisable to ascertain whether this might be done if diabetic (depancreatized) animals were employed. It was considered possible that failure to demonstrate increased retention of sugar in the muscles or liver, as a result of insulin, might depend solely

on the fact that the method used was not sensitive enough to reveal it. Since there is no doubt not only that sugar is abundantly deposited as glycogen in the liver of diabetic animals, but also that its combustion is accelerated in the muscles when insulin is given, we decided to test the reliability of the method in this way. The following results were obtained.

Experiment 1. Dog, 8.75 kgm. Depancreatized April 19, 1923. Etherized on April 24 and cannulae placed in femoral artery and inferior vena cava.

TIME	SUGAR PER 100 CC. BLOOD		
	Femoral artery	Femoral vein	Difference F.A.-F.V.
	mgm.	*mgm.*	*mgm.*
11:12 a.m.	373	384	
11:40 a.m.	368	365	3
12:05 p.m.	383	Clot	
12.06 p.m.	40 units insulin		
12:44 p.m.	414	409	5
1:40 p.m.	430	418	12
	60 units insulin		
2:40 p.m.	460	442	18
2:47 p.m.	60 units insulin		
3:35 p.m.	472	443	29
3:40 p.m.	60 units insulin		
4:55 p.m.	450	430	20

The most remarkable result of the experiment is the inability of insulin to cause any reduction in the blood sugar of either vessel, a result which is probably to be attributed to the etherization. In spite of the fact that 220 units (clinical) were injected the blood sugar steadily rose and although the difference between the femoral artery and vein became somewhat greater toward the end of the experiment, this cannot be considered as significant. The liver was found to contain 0.09 per cent of glycogen and 25.0 per cent of fat. The small trace of glycogen does not indicate that insulin had succeeded in causing glycogenesis. The ether probably prevented it.

Experiment 2. Dog, 15 kgm. Depancreatized April 30, 1923. Etherized at 9:45 a.m. May 4, 1923. Cannulae placed in the saphenous and mesenteric veins and the carotid artery. The liver contained only a trace of glycogen but 32.9 per cent of fat. The muscles contained 0.1 per cent of glycogen.

In this experiment practically all of the blood analyses were conducted in duplicate and the results corresponded closely. It will be observed, as in experiment 1, that insulin had only a very slight effect and that the

TIME	SUGAR PER 100 CC. BLOOD			
	Carotid artery	Saphenous vein	Difference C.A.–S.V.	Mesenteric vein
	mgm.	*mgm.*	*mgm.*	*mgm.*
9:50 a.m.	200			
9:55 a.m.		220		
10:10 a.m.	313	228	85	
10:25 a.m.	329			325
10:30 a.m.		322		
10:35 a.m.	190 units (clinical) of insulin			
10:50 a.m.	333	312	21	
10:55 a.m.				330
11:00 a.m.				340
11:15 a.m.		310		
11:17 a.m.	331		21	
11:21 a.m.				306
11:48 a.m.		282	30	
11:50 a.m.	312			285
12:35 p.m.	302			

differences between the blood sugars were of the usual magnitude. The absence of glycogen in the liver indicates that no glycogenesis had occurred.

Experiment 3. Dog, 5.5 kgm. Depancreatized May 8. Etherized 9:55 a.m. on May 11. Sugar in blood of saphenous vein just prior to etherization, 0.317 per cent. Cannulae in mesenteric vein and carotid artery for collection of blood, but from the femoral vein this was done by means of a hypodermic needle.

TIME	SUGAR PER 100 CC. BLOOD			
	Carotid artery	Femoral vein	Difference C.A.–F.V.	Mesenteric vein
	mgm.	*mgm.*	*mgm.*	*mgm.*
10:15 a.m.	312, 307 } 309			315, 307 } 312
10:16 a.m.		297	12	
10:35 a.m.	327			305
10:36 a.m.		305	22	
10:42 a.m.	150 units insulin			
10:50 a.m.	150 units insulin			
10:52 a.m.	135 units insulin			
11:00 a.m.	298			280
11:02 a.m.		275	23	
11:20 a.m.	231			220
11:22 a.m.		215	16	
11:45 a.m.	205	198	7	

The liver contained 0.07 per cent of glycogen and 15.6 per cent of fat. The heart contained 0.65 per cent glycogen and the muscles 0.14 per cent. In this experiment it was possible to cause decided reduction in blood sugar but only by administering enormous doses of insulin. Comparison of the percentages of sugar in the various bloods does not show that the usual differences have become significantly changed as a result.

Experiment 4. Dog, 9 kgm. Depancreatized May 10. Etherized at 2:17 p.m. on May 14, the blood sugar in the saphenous vein prior to etherization being 0.560 per cent. Cannulae placed in femoral artery and saphenous and femoral veins.

TIME	SUGAR PER 100 CC. BLOOD			
	Femoral artery	Femoral vein	Difference F.A.-F.V.	Mesenteric vein
	mgm.	*mgm.*	*mgm.*	*mgm.*
2:40 p.m.	585	561	24	
2:55 p.m.	647	615	32	592
3:00 p.m.	470 units insulin subcutaneously			
3:12 p.m.	580	581	1	
3:28 p.m.	562	590		

Although this experiment is a comparative failure it is reported since it does show a slight reduction in the hyperglycemia following an enormous dose of insulin. The reduction is not accompanied by evidence of sugar retention by the muscles, on the contrary the muscles appear to be retaining less sugar than before insulin was given.

These experiments demonstrate very clearly that insulin has practically no hypoglycemic action in etherized depancreatized animals. In etherized normal animals although large amounts of insulin are also required the blood sugar can always be brought down to, or below, the convulsive level, as the following experiment will illustrate:

Experiment 5. Dog, 6.2 kgm. Etherized 10:40 a.m. One hundred and twenty-five units and later 100 units of insulin were injected, reducing the blood sugar from 0.263 per cent of 0.040 per cent. In this experiment the percentage of lactic acid was also determined in the arterial blood and was found to become somewhat increased but not significantly so. This failure of insulin to cause reduction of the blood sugar in etherized diabetic animals renders the experiments of no value for the purpose for which they were performed, namely, to see whether a more marked difference between the percentages of sugar in the arterial and venous blood could be demonstrated as a result of insulin. The unexpected persistence of the hyperglycemia in spite of large doses of insulin is clearly due to the presence of ether in the body. This seems entirely to annul the hypoglycemic action of insulin in pancreatic dia-

	TIME	MGM. SUGAR PER 100 CC. BLOOD
1	11:00 a.m.	Blood 263
	11:05 a.m.	Blood for lactic acid 19
2	11:10 a.m.	Blood 252
3	11:20 a.m.	Blood 252
4	11:20 a.m.	Blood 234
	11:22 a.m.	5 cc. insulin. 25 units/ cc. Rectal temperature 39°C.
5	11:48 a.m.	Blood 182. Rectal temperature 39°C.
6	12:15 p.m.	Blood 128
7	12:35 p.m.	Blood 107. Rectal temperature 38.5° C.
8	1:00 p.m.	Blood 076
9	1:00 p.m.	Blood 083
	1:00 p.m.	Bloods 3 and 4, lactic acid, 0.033 per cent
	1:05 p.m.	5 cc. insulin. Approx. 20 units/ cc. Rectal temperature 39°C.
10	1:55 p.m.	Blood 052. Rectal temperature 40°C.
11	2:10 p.m.	Blood 040

betes although it has the desired effect in the various forms of nervous and asphyxial hyperglycemia and in that due to epinephrin (8). We can offer no explanation for this result. Its practical importance in connection with the administering of anesthetics to diabetic patients will, however, be apparent. This demonstration of the influence of ether may account for the complete failure to find experimental conditions favorable to the demonstration of increased retention of sugar by the liver or muscles as a result of insulin in other than depancreatized animals. A few observations were therefore undertaken using a decerebrate preparation (dog), but with unsatisfactory results. In one of these experiments, for example, the blood sugar percentages immediately before and in one-half and one hour after injecting insulin were as follows:

	BEFORE INSULIN	ONE-HALF HOUR AFTER INSULIN	ONE HOUR AFTER INSULIN
Pancreatic duodenal vein	0.162	0.134	0.107
Femoral artery	0.179	0.150	0.145
Femoral vein	0.159	0.128	0.144

Although in one-half hour there is some evidence of sugar retention by the muscles, this was not confirmed in the results of one hour.

Finally, we have performed two observations on dogs anesthetized with amytal (iso-amyl-ethyl-barbituric acid) a narcotic which, as Page (5) has shown in rabbits, causes no increase in blood sugar. We have found that in dogs also this narcotic causes complete loss of consciousness and muscular relaxation unaccompanied by any rise in blood sugar, indeed, if anything, by a slight fall. Insulin both in rabbits (Page) and in dogs has its usual hypoglycemic action. The following experiments were performed.

Experiment 1. Dog, 9 kgm. (11 tablets "amytal" injected subcutaneously at 12:30 p.m. At 2:30 p.m. the animal was quite unconscious.

TIME	SUGAR PER 100 CC. BLOOD	
	Femoral artery	Femoral vein
	mgm.	*mgm.*
2:35 p.m.	83	83
2:50 p.m.	80	81
2:55 p.m.	25 units (clinical) insulin	
3:40 p.m.		80
4:00 p.m.	70	80
4:20 p.m.	58	58
4:30 p.m.	10 units (clinical) insulin	
4:50 p.m.	48	58
6:50 p.m.	77	70
8:20 p.m.	57	64

In most observations the results are higher for the vein than the artery and at no stage is there any evidence that the muscles are retaining more sugar. The experiment was not, however, considered satisfactory partly because blood was not taken from the portal vein and vena cava and partly because some unusual difficulties were encountered in obtaining clear filtrates in the blood samples.

Experiment 2. March 13, 1924. Dog. Injected with "amytal" about 2 hours previously, the animal being deeply narcotized. A long glass cannula was inserted in the central end of the left renal vein so that its free end lay opposite the hepatic veins and ligatures placed loosely around the vena cava below and above the level of the free end of the cannula. A similar cannula was placed in the central end of the pancreatico duodenal vein. As the blood samples were removed from the vena

TIME	SUGAR PER 100 CC. BLOOD			REMARKS
	Portal vein	Vena cava	Difference P.V.–V.C.	
	mgm.	*mgm.*	*mgm.*	
4:20 p.m.	114			
4:30 p.m.	114	113	1	
4:40 p.m.	135	130	5	Sugar being in intestine
4:44 p.m.	170	155	15	
4:55 p.m.	177	170	7	
5:03 p.m.	176	174	2	Sugar in intestine insulin subcutaneous
5:25 p.m.	159	148	11	
5:45 p.m.	137	134	3	

cava the loose ligatures were pulled tight so as to shut off the portion of the vein in which the free end of the cannula lay.

At 3:31 p.m., 100 cc. of a 5.5 per cent solution of glucose were placed in a long loop of intestine and this was repeated at 4:50, at the same time as 20 units of insulin were injected subcutaneously. The experiment proceeded satisfactorily in every way except that there was some difficulty in obtaining perfectly clear filtrates in certain of the blood samples. (This, by the way, we find not infrequently to be the case when Folin-Wu precipitants are employed.) The estimations were made in duplicate, the results recorded being the average of those which checked to within 2 per cent. This was the case with all results after 4:44 and in the results preceding this time, when the duplicates deviated by more than 5 per cent the lowest result in each case is given. It will be seen that the retention of sugar by the liver, if anything, fell off some time after the effect of insulin had begun to show itself. Taking these two experiments together we consider the results offer no encouragement to a further investigation of the problem by the method of comparison of the blood sugar in different vessels.

Discussion. We had intended withholding these results, some of which were obtained two years ago, until we found an opportunity to observe the effects of insulin on the hyperglycemia caused by continuous intravenous injections of glucose. This experiment has not been possible and we have decided to publish the results as they stand because other workers have meanwhile concluded, by the use of similar methods, that increased disappearance of glucose can be demonstrated both in the liver and muscles. Cori, Cori and Goltz (6), for example, claim to have evidence that insulin causes a diminished output of sugar by the liver and a larger intake by the muscles. The methods which they employed have the great advantage over ours in that the blood samples were collected in rabbits without narcosis.

In a preliminary operation, the day before that of the actual experiment, an abdominal window was made and the falciform ligament of the liver cut. This allowed the viscus to fall away from the diaphragm when the animal was placed at an angle of 50° to the table. Blood was removed from one of the four hepatic veins by a syringe with a special needle bent at its end, introduced through the previously prepared abdominal window. Both for this operation and for removing samples of blood from the femoral vessels or the neck vein, narcosis was not required.

In eight rabbits the average difference between the hepatic vein and neck vein was found to be 28 mgm. per 100 cc. blood, (minimum 24, maximum 33). In the five experiments recorded in which insulin was injected the above difference was decidedly less (i.e., below the minimum for injected animals) in 2, but no different or greater in 3. In the table

which shows the most marked decrease (no. 2) only 1 unit of "iletin" had been injected one hour previously whereas in the other 4 cases of this group 10 units were used, the reason given being that it was found that the hypoglycaemic effects of the particular sample of insulin used were unusually feeble on normal rabbits. This leaves only one experiment (table 4) in which the results indicate that insulin had depressed the glycogenolytic process but even this is uncertain since the results might equally well be explained as due to a *lessened* retention of glucose in the vascular area drained by the neck veins. No observations could be made of changes in blood pressure and it is well known that these may be responsible for considerable alterations in blood sugar.

The normal average difference between the blood sugar in the leg artery and vein is given as 8 mgm. per 100 cc. blood (20 observations) the maximum being 13 and the minimum 3. Seven experiments are recorded in which these comparisons were made following insulin. In 3 of these the maximum difference found in normal animals was exceeded. In the first of these (table 8) this difference was 17 and it was observed both in one and one-half hours, when the blood sugar in the vein stood at 42 mgm. per cent, and in three hours when the latter showed 53 mgm. per cent. One the other hand in the second case (table 10) the observed percentages in artery and vein were strictly within the normal limits until over 3 hours following insulin. In the third case (table 11) the excessive difference occurred one and one-half hours after insulin. In all other recorded cases the differences were within the normal limits or, indeed, below it in one case in which also the degree of hypoglycemia was the most marked of all the experiments.

In 9 cases the sugar was compared in the blood of the hepatic vein and femoral artery and the average difference was 23 mgm. per cent in favor of the former (maximum 26, minimum 20). Five experiments are recorded in which insulin was given. These were the last experiments to be performed and the conclusions are "that several types of insulin action can be distinguished"—"both the liver and the muscle are influenced simultaneously" or "the liver alone seems to be responsible" or "the muscle alone is influenced." Such varied types of results do not appear to us to justify the conclusions which the authors have drawn from them and they can scarcely serve to cast much light on the problem of the mechanism of the hypoglycemic action of insulin.

In passing, we would call attention to the marked difference found by these workers between the sugar content of blood from the liver vein and that from the femoral artery, viz., an average of 0.023 per cent more in the former. This is exactly the opposite relationship to that found by Lepine and Boulud (7) between the sugar in blood samples taken simultaneously from the carotid artery and right ventricle.

CONCLUSIONS

1. We have been unable to demonstrate increased disappearance of sugar from the blood into the muscles, as the result of insulin, by comparison of the sugar concentration in the arterial and venous blood of muscles of anesthetized animals.

2. We have been unable, under the same conditions, to demonstrate increased disappearance of sugar in the liver.

3. Insulin is practically without any hypoglycemic effect on the blood sugar of depancreatized animals under deep ether anesthesia.

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THE COMPARATIVE ACTION OF PANCREATIC SECRETIN WHEN INJECTED INTO A SYSTEMIC ARTERY, SYSTEMIC VEIN, AND THE PORTAL CIRCULATION[1]

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Djenab (4) has reported experiments in which pancreatic secretin injected into either the portal vein or the femoral artery proved to be rather ineffective or even impotent; introduction of the same secretin preparation into a systemic vein such as the saphenous or the femoral, was followed by the characteristic prompt flow of pancreatic juice. These results were interpreted as meaning that the muscle and liver capillaries either hold back temporarily or destroy the hormone; in the case of the liver it was suggested that this organ by so acting protected the pancreas against secretory fatigue. These results were also regarded as favoring the Bickel theory of the mechanism of control of pancreatic secretion, which theory attributes the continuous flow of juice from the various digestive glands to humoral influences on the neuro-glandular apparatus, while the discontinuous character of secretion is regarded as brought about by inhibitory or secretory stimuli sent to the glands along the extra-glandular nervous system.[2]

Djenab gave no protocols in his paper and contented himself with more or less general statements which, in the absence of even a representative detailed experiment, are not very convincing. The few details that are presented are not sufficient to enable the reader to evaluate properly the conclusions that are drawn. Perhaps this may be excused if the paper be regarded as a preliminary communication rather than as a detailed exposition of the experiments performed. A search of the literature has failed to disclose a further publication of these experiments *in extenso*.

[1] A preliminary report of these experiments was made at the XIth International Physiological Congress, Edinburgh, Scotland, July, 1923. The expenses of this investigation were defrayed in part by a contribution from the Russell H. Chittenden Research Fund for Physiological Chemistry.

[2] This statement of Bickel's hypothesis is based on Djenab's presentation; we have not seen any statement of this in such of Bickel's own published work as has been accessible to us.

Inasmuch as either the complete loss or marked diminution in potency of secretin when passed through the liver capillaries really calls for an explanation and throws doubt upon the whole hormone hypothesis with respect to the pancreas, a further examination of this question experimentally seems worth while.

Only a very few of the host of investigators who have studied the secretin problem have ever concerned themselves with the question of the effectiveness of the hormone when passed into the body and to the pancreas along the path that must be followed under normal physiological conditions, namely, the portal system.

Matsuo (9), in his first attempts at cross circulation experiments designed to test the secretin hypothesis, endeavored to join his animals in a portal-to-jugular fashion but was unsuccessful. Later attempts where the union was carotid-to-jugular resulted in success. By his line of experimentation, therefore, it is quite clear that this investigator recognized the portal entry as the normal one and planned his experiments accordingly. A comparison of the portal with other possible channels was not made, however, this author having assumed, like nearly all other workers on the secretin problem, that there would be no differences in the secretin effects obtained whether the active principle reached the pancreas after first passing through the liver or not.

Halliburton and de Souza (5) appear to have been the only other investigators who have interested themselves in this question with reference to pancreatic secretin. They found that the effect of secretin was distinct but less, when the injection was made into the portal vein or femoral artery, than that resulting from an injection into the femoral vein. In their opinion the extra dilution of the active principle afforded by the passage through capillaries is sufficient to account for the different results obtained. Our own experiments, about to be described, certainly support this conclusion with reference to the arterial path; with respect to the portal entry this explanation is perhaps not quite satisfactory.

Some appreciation of the possible rôle of the liver in handling *gastrin* substances has appeared recently in the work of Matsuoka (10), Lim and Ammon (7) and Ivy and McIlvain (6). Matsuoka, working in Bickel's laboratory, experimented with histamine and some of the "secretin" (gastrins as understood by English and American writers) which Bickel and his students have shown to be produced from spinach and other materials by heating to 130°C. with strong acid for a short period. The results are stated as confirming those of Djenab, and the interpretation placed upon them is that the liver acts as a selective agent with respect to the gastrin materials contained in such a mixture as an extract of hydrolyzed spinach must be, and allows them to pass through. Lim

and Ammon found that the activity of gastrin when introduced by the portal vein was less than that of the same preparation when injected into the femoral vein.

Ivy and McIlvain (6) have come about as close to duplicating by experimental technic what must occur normally, as it is possible to do, but it should be noted that these investigators were studying a gastrin and not the pancreatic secretin problem. In their experiments, the substance being tested was placed in a Thiry intestinal fistula and the flow of gastric juice from a Pawlow stomach pouch in the same animal noted. Now in order to test a preparation of pancreatic secretin in an unanesthetized animal, it would be necessary to devise some arrangement by which the discharge of acid chyme into the duodenum during the period of the test would be prevented. A temporary blockage of the pylorus might be accomplished by means of an inflated balloon using the procedure adopted by Popielski (15) or perhaps in other ways. Experiments utilizing such a technic we have as yet been unable to perform. However, in view of the few comparisons that have been made of the activity of pancreatic secretin when introduced by the various possible channels, it was decided to experiment along this line using the secretin technic that has been employed since the work of Bayliss and Starling (1). The results obtained were so clear-cut that we believed them to be worthy of report.

Experimental. Dogs were used as experimental animals. In each case, the anesthetization consisted of a preliminary subcutaneous injection of a small amount of morphine—not over 5 mgm. per kilo—followed by ether insufflation. That this amount of morphine does not exert any inhibitory effect on the pancreas appears to be demonstrated by the results obtained. A ligature was placed around the pylorus to prevent discharge of acid chyme from the stomach, a cannula inserted into the larger pancreatic duct, and injection cannulae placed in the femoral vein on one side, femoral artery in the opposite leg, and a branch of the splenic vein, the last for injections into the portal circulation.

A variety of secretin solutions was tested; some were prepared by HCl extraction of intestinal mucosa that had previously been treated with boiling alcohol to remove the depressor substance, and then dried; others were made by acid extraction of the fresh mucosa. No differences in results that could be attributed to the source or the method of preparation of secretin were obtained.

SYSTEMIC ARTERY VERSUS SYSTEMIC VEIN. *Pancreatic secretin preparations, when injected into a systemic artery, have approximately the same potency as when injected into a systemic vein; the greater dilution of the active principle which must occur when it enters via an artery seems sufficient to account for the slightly different results obtained.* The following two protocols are typical of many and justify the above statement.

PROTOCOL. *Experiment 2.*

(a)	1:00–1:01	20 cc. pig secretin[3] into left femoral vein
	1:00–1:05	Cannula filling with pancreatic juice
	1:05–1:10	10 drops pancreatic juice
	1:10–1:15	8 drops pancreatic juice
	1:15–1:20	3 drops pancreatic juice
	1:20–1:25	1 drop pancreatic juice
(b)	1:25–1:26	15 cc. pig secretin—same as (a)—into left femoral vein
	1:25–1:30	15 drops pancreatic juice
	1:30–1:35	10 drops pancreatic juice
	1:35–1:40	3 drops pancreatic juice
	1:40–1:45	1 drop pancreatic juice
(c)	1:45–1:46	15 cc. pig secretin—same as (a)—into right femoral artery
	1:45–1:50	18 drops pancreatic juice
	1:50–1:55	8 drops pancreatic juice
	1:55–2:00	2 drops pancreatic juice
	2:00–2:05	1 drop pancreatic juice
(d)	2:05–2:06	10 cc. dog secretin[3] into left femoral vein
	2:05–2:10	3 drops pancreatic juice
	2:10–2:15	2 drops pancreatic juice
	2:15–2:20	0 drop pancreatic juice
	2:20–2:25	1 drop pancreatic juice
(e)	2:25–2:27	10 cc. dog secretin—same as (d)—into right femoral artery
	2:25–2:30	0 drop pancreatic juice
	2:30–2:35	2 drops pancreatic juice
(f)	2:35–2:37	20 cc. dog secretin[3]—not same as (d)—into left femoral vein
	2:35–2:40	8 drops pancreatic juice
	2:40–2:45	4 drops pancreatic juice
	2:45–2:50	1 drop pancreatic juice
(g)	2:50–2:51	20 cc. dog secretin—same as (f)—into right femoral artery
	2:50–2:55	3 drops pancreatic juice
	2:55–3:00	2 drops pancreatic juice
	3:00–3:05	1 drop pancreatic juice
(h)	3:05–3:06	14 cc. pig secretin—same as (a)—into left femoral vein. Final control
	3:05–3:10	13 drops pancreatic juice
	3:10–3:15	4 drops pancreatic juice
	3:15–3:20	1 drop pancreatic juice
	3:20–3:25	0 drop pancreatic juice
	3:25–3:30	0 drop pancreatic juice
	3:30–3:35	0 drop pancreatic juice

PROTOCOL. *Experiment 3.*

(a)	2:25–2:27	10 cc. pig secretin[4] into left femoral vein
	2:25–2:30	Cannula fills and 17 drops of pancreatic juice
	2:30–2:35	28 drops pancreatic juice
	2:35–2:40	8 drops pancreatic juice
	2:40–2:45	2 drops pancreatic juice
	2:45–2:50	0 drop pancreatic juice

[3] By 0.4 per cent HCl extraction of intestinal mucosa previously treated with hot alcohol and dried.

[4] By 0.4 per cent HCl extraction of intestinal mucosa previously treated with hot alcohol and dried.

(b)	2:50–2:52	10 cc. pig secretion—same as (a)—into right femoral artery
	2:50–2:55	23 drops pancreatic juice
	2:55–3:00	13 drops pancreatic juice
	3:00–3:05	3 drops pancreatic juice
	3:05–3:10	0 drop pancreatic juice
(c)	3:10–3:12	10 cc. dog secretin into left femoral vein
	3:10–3:15	13 drops pancreatic juice
	3:15–3:20	4 drops pancreatic juice
	3:20–3:25	2 drops pancreatic juice
	3:25–3:30	0 drop pancreatic juice
	3:30–3:35	1 drop pancreatic juice
(d)	3:35–3:36	10 cc. dog secretin—same as (c)—into right femoral artery
	3:35–3:40	7 drops pancreatic juice
	3:40–3:45	3 drops pancreatic juice
	3:45–3:50	1 drop pancreatic juice
(e)	3:50–3:51	10 cc. pig secretin—same as (a)—into left femoral vein. Final control
	3:50–3:55	30 drops pancreatic juice
	3:55–4:00	8 drops pancreatic juice
	4:00–4:05	1 drop pancreatic juice
	4:05–4:10	2 drops pancreatic juice

These results are so clear-cut that a discussion of them hardly seems necessary beyond pointing out that the slight differences in potencies obtained may be explained equally well by a dilution of the active principle when injected into an artery, as to assume with Djenab that the muscle capillaries hold back or destroy secretin.

PORTAL SYSTEM VERSUS SYSTEMIC VEIN. *Pancreatic secretin, when injected in single large doses into the portal system, is distinctly weaker in its action on the pancreas and effective after a much longer latent period than when injected via a systemic vein.* The following protocol, typical of many, illustrates this fact.

PROTOCOL. *Experiment 4.*

(a)	3:50–3:51	10 cc. pig secretin—same as (a) experiment 3—into left femoral vein
	3:50–3:55	30 drops pancreatic juice
	3:55–4:00	8 drops pancreatic juice
	4:00–4:05	1 drop pancreatic juice
	4:05–4:10	2 drops pancreatic juice
(b)	4:10–4:11	10 cc. pig secretin—same as (a)—into splenic vein
	4:10–4:15	0 drop pancreatic juice
	4:15–4:20	4 drops pancreatic juice
	4:20–4:25	1 drop pancreatic juice
	4:25–4:30	2 drops pancreatic juice
(c)	4:30–4:32	10 cc. pig secretin—same as (a)—into splenic vein
	4:30–4:35	0 drop pancreatic juice
	4:35–4:40	5 drops pancreatic juice
	4:40–4:45	3 drops pancreatic juice
	4:45–4:50	2 drops pancreatic juice
	4:50–4:55	1 drop pancreatic juice

(d)	4:55–4:56	10 cc. dog secretin[5] into splenic vein
	4:55–5:00	0 drop pancreatic juice
	5:00–5:05	1 drop pancreatic juice
	5:05–5:10	1 drop pancreatic juice
(e)	5:10–5:14	34 cc. dog secretin—same as (d)—into splenic vein
	5:10–5:15	0 drop pancreatic juice
	5:15–5:20	14 drops pancreatic juice
	5:20–5:25	8 drops pancreatic juice
	5:25–5:30	2 drops pancreatic juice
	5:30–5:35	2 drops pancreatic juice
(f)	5:35–5:37	25 cc. dog secretin[5]—not same as (d)—into splenic vein
	5:35–5:40	1 drop pancreatic juice at 5:39
	5:40–5:45	4 drops pancreatic juice
	5:45–5:50	2 drops pancreatic juice
	5:50–5:55	1 drop pancreatic juice
(g)	5:55–5:57	25 cc. dog secretin—same as (f)—into left femoral vein
	5:55–6:00	23 drops pancreatic juice
	6:00–6:05	7 drops pancreatic juice
	6:05–6:10	2 drops pancreatic juice
	6:10–6:15	0 drop pancreatic juice
(h)		Period of observation of normal flow
	6:15–6:20	1 drop pancreatic juice
	6:20–6:25	1 drop pancreatic juice
	6:25–6:30	2 drops pancreatic juice
	6:30–6:35	2 drops pancreatic juice

The characteristic thing about the portal injection of pancreatic secretin, when contrasted with the usual injection into a systemic vein, is the longer latent period which occurs before the pancreatic juice begins to flow. In some of our earlier experiments in which relatively weak secretin preparations were tested, this longer latent period was followed by such a slight flow of pancreatic secretion as to raise a question whether the flow being observed really could be attributed to the secretin that had been injected. It is quite possible that some of the failures—so-called—of Djenab to demonstrate the stimulatory action of secretin via the portal vein were due to the use of a very weak secretin preparation. Repeated experimentation to determine whether this longer latent period is a true characteristic of the action of secretin when passed through the liver and not an experimental artifact, has satisfied us that when single large doses of secretin are employed, under the conditions set up in our experiments, the liver does prevent them from acting as promptly upon the pancreas as they do when put directly into the extrahepatic circulation.

Experiments, recently completed by Plummer, Deuel and Cowgill (12) in which secretin solutions were introduced by the systemic and portal routes at very slow rates, have shown that the liver must absorb secretin

[5] By 0.4 per cent HCl extraction of intestinal mucosa previously treated with hot alcohol and dried.

to what may be regarded as a saturation point; when this is reached, subsequent injections of this hormone by the portal route more nearly simulate the results obtained by the systemic pathway. Lim and Ammon (7) suggested that this might be true but offered no convincing experimental evidence in support of it.

It seems pertinent to call attention to the fact that the latent period following the introduction of acid into the intestine more nearly approximates that which characterizes the action of secretin when injected into the portal system. Recently Luckhardt, Henn and Palmer (8) raised anew the question as to the specificity of the pancreatic secretin[6] obtained by acid extraction of intestinal mucosa; one of their arguments is based upon a comparison of the effect of such extracts with that obtained by putting hydrochloric acid into the gut. A careful reading of their report will disclose the fact that their preparations were never injected via the portal circulation and the liver thereby given a chance to do what it probably does under normal physiological conditions with secretin or gastrin substances coming to it from the intestines. We have compared the effect of secretin via the portal vein with that obtained when acid is introduced into the duodenum and have noticed that in both cases there is a latent period much longer than that which characterizes secretin via the femoral vein. The following extract from one of our protocols illustrates this similarity.

Extract from protocol of Dog 104

	Observation of normal flow of pancreatic juice
3:41–3:46	1 drop pancreatic juice
3:46–3:51	1 drop pancreatic juice
3:51–3:53	15 cc. 0.4 per cent HCl into intestine
3:51–3:56	1 drop pancreatic juice at 3:56
3:56–4:01	6 drops pancreatic juice
4:01–4:06	1 drop pancreatic juice
4:06–4:11	1 drop pancreatic juice
4:11–4:16	0 drop pancreatic juice
4:16–4:21	1 drop pancreatic juice

When much larger amounts of hydrochloric acid are placed in the intestine, the latent period is somewhat shorter, but never as brief as that which characterizes an injection of secretin into the femoral vein. This suggests that the liver behaves in the same way with respect both to the secretin substance contained in an acid extract of intestinal mucosa and to the hormone when secreted under the stimulus of acid present in the

[6] This question has been debated for many years and the arguments offered by Luckhardt and his collaborators based upon the secretin-like action of extracts of different tissues have been made before, although not in precisely the same form, by many other investigators, notably Borissow and Walther (3), Popielski (13), (14), Modrakowski (11) and others. A good review of this work is contained in Babkin's monograph (2).

duodenum; and to this extent one is justified for the present in adopting an open-minded attitude with respect to the question raised by Luckhardt and his collaborators.

There is current in the literature what appears to us to be an ill-advised use of the word secretin. The German workers, chiefly Bickel and his pupils, use this word to mean any substance of unknown chemical nature that stimulates a gland to secrete, and therefore write concerning gastrin and pancreatic secretin materials without distinguishing between them except by context. There may be etymological reasons justifying this course, but in view of the length of time during which secretin has always signified a substance, hormone in nature, that promotes the flow of *pancreatic* juice—Bayliss and Starling suggested the word secretin 1902—and in view of the later appearance of the word *gastrin* to designate the hormone acting upon the gastric glands, it seems ill-advised to extend the term secretin so as to include gastrin substances as well. If a new nomenclature is necessary, it would seem better to introduce the term *pancreasin*, or some other word of similar significance to specify the stimulatory hormone that acts upon the pancreas.

SUMMARY—CONCLUSION

Pancreatic secretin preparations were tested upon anesthetized animals following the usual secretin technic, comparisons being made of secretin injected via the femoral vein, femoral artery and the portal circulation. The result obtained with secretin via the artery did not differ in any striking way from that obtained following the injection into the vein. When secretin is introduced into the portal circulation, in single large doses as contrasted with the femoral vein, a greatly reduced potency and a much longer latent period characterize its action upon the pancreas. Whereas a venous injection of secretin ordinarily affects the pancreas after from fifty to sixty seconds, from three to five minutes elapse before an injection via the portal system is followed by an increased flow of pancreatic juice. This longer latent period is similar to that which follows the introduction of acid into the duodenum. These facts indicate that the portal path of absorption should receive due attention by investigators engaged in a study of the physiology of secretin, gastrin and similar substances, in order to make the experimental conditions approximate more closely those which prevail in the normal organisms.

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THE INDUCTION OF A SEXUALLY MATURE CONDITION IN IMMATURE FEMALES BY INJECTION OF THE OVARIAN FOLLICULAR HORMONE

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The attainment of sexual maturity of the female mammal depends upon the endocrine function of the ovary but it has not been clearly demonstrated to which part of the gonad this is due. In a recent review Marshall (1) writes "It is not known what precise ovarian elements are responsible for the production of the hormone which is an excitant for the development of the female organs and characters or the possible inhibition of the distinctively male ones, ... "

Although the physiology of the immature ovary is still quite obscure, it is well established that the first ovulation does not usually occur until puberty (2). Consequently no follicles attain to full maturity until that time, and there are normally no corpora lutea present in the immature ovary. Therefore the follicles and interstitial tissue are the only two possible sources of the hormone which brings about a sexually mature condition in the female.

Ovarian interstitial tissue, used as a general term implying a possible endocrine function, is not yet on a firm histological basis. Several investigators have failed to find it in appreciable amounts in mammalian ovaries of several species. It has, however, been especially stressed by Steinach (3) as a source of production of the hormone which brings about sexual maturity in the female. Marshall (p. 346) summarizes this conception:

> According to Steinach and his followers, the interstitial cells of the ovary represent the "puberty gland" of the female organism, that is to say, that gland which is responsible for all the essentially female characteristics including not only the accessory generative organs such as the uterus and mammary glands but also the secondary characters of sex and the psychological female characters. The organ is called the "puberty gland" because those characters which depend upon its activity undergo marked development at puberty.

It may have seemed logical to stress the interstitial tissue of the ovary as the "puberty gland" when it was considered that ova lie dormant in the ovary until puberty. There is some evidence, however, that follicles in the immature ovary are actively growing (4, p. 440), but that their full development is in some way restrained. If this is so, are they also elaborating the hormone which causes sexual development?

The extraction of the follicular hormone from large follicles of ovaries of swine and the demonstration that it will substitute satisfactorily for the hormonal function of the adult ovary in producing maximum oestrous growth and function of the genital tract in rats and mice (5), (6), (7)has led us to test its effect upon immature animals.

Immature rats of ages ranging from the time of weaning (24 days) to the age of 54 days have been used in these experiments. The extensive monograph by Long and Evans furnishes reliable data concerning the time of the attainment of puberty in these animals. This time varies greatly; considerable numbers of animals being included in a range between 40 and 90 days of age. The mode of the curve is 76.5 days.

The work of Long and Evans shows further that two of the principal criteria of sexual maturity need not necessarily be coincident. The vagina of the immature rat is completely closed, its external one-third being a solid cord of cells. The opening of the vaginal orifice and the appearance of the first signs of oestrus may therefore be applied to these animals as one definition of sexual maturity. The ovaries of animals killed during the next few days, however, often do not contain corpora lutea, thus indicating that ovulation has not occurred. From an extended series of animals Long and Evans conclude that the first ovulation usually occurs about five days (the duration of one cycle) after the opening of the vagina. Consequently this might also be defined as the time of attainment of puberty.

Since in this paper we are interested chiefly in the actual substitution of the follicular hormone for the endocrine function of the ovary, and since some of our experiments involve the removal of both ovaries, the choice of the first definition of sexual maturity, the opening of the vagina and first signs of oestrus, seems more applicable. Therefore reference to a sexually mature condition in this paper refers to the condition of the genital tract and not to the production of ova.

EXPERIMENTAL RESULTS. The follicular contents (liquor folliculi, some follicle cells and occasional ova) from hog ovaries selected for advanced follicular development, or active extracts of this material (7), were used in these experiments. The material injected contained from 3 to 20 "rat units" of the follicular hormone. A rat unit is the minimal quantity which will induce full oestrous growth in the genital tract of a spayed adult rat 48 hours after the first of three 1 cc. injections given at intervals of 4 to 6 hours (6 b).

Several immature females of approximately the same weight were chosen from large litters, differentiated by ear notches, and kept in the same cage. One from each litter served as a control. The others were given varying numbers of injections of the follicular hormone. The first series were normal animals, the last spayed animals.

In table 1 are listed the data on the first series of injections. The age range of the animals used is from 25 to 54 days. Litter sisters are grouped together. Liquor folliculi and concentrated alcoholic extract of follicular contents were given in 1 cc. injections at quite closely spaced intervals.

TABLE 1

Effect of injections of the follicular hormone upon normal immature rats, 25 to 54 days of age (1st series)

ANIMAL	AGE	MATERIAL INJECTED	INJECTIONS					RESULTS 4/25 9 a.m. VAGINA OPEN, OESTRUS SMEAR
			4/23		4/24			
			1:30 p.m.	8:50 p.m.	2:40 a.m.	6:30 a.m.	12 m.	
	days							
1	25	L.F.*	1 cc.	1	1	1	0.7	+‡
1-C	25	Control	/	/	/	/	/	−
2	30	12 Alc.†	1 cc.	1	1	1	0.6	+
2-C	30	Control	/	/	/	/	/	−
3	47	L.F.	1 cc.	1	1	1	1	+
3-C	47	Control	/	/	/	/	/	−
4	54	12 Alc.	1 cc.	1	1	1	0.6	+
4-N	54	L.F.	1 cc.	1	1	1	1	+§
4-C	54	Control	/	/	/	/	/	−

* L.F. = liquor folliculi from large follicles of hog ovaries.

† 12 Alc. = alcohol soluble substance from follicular contents.

‡ Animal 1 was sick on the second day of injections, the dose being much too large.

§ The vagina was open at 12 noon on 4/24, too soon to have been the result of injections.

An open vaginal orifice was noted and an oestrous smear taken from animal 4N at noon on the 24th, 22½ hours after the first injection. Since the oestrous smear depends upon growth of the vaginal epithelium to maximum thickness and, as shown in our earlier experiments (6b), this requires from 36 to 48 hours in the spayed adult rat, it is probable that rat 4N was on the verge of puberty when injections were begun. The litter sister, 4C, did not attain to sexual maturity during the course of the experiment.

At 9 a.m. on the 25th a congested vulva and open vagina were noted in animals 2, 3 and 4, and smears made at this time indicated the establishment of a typical oestrous period (details of the tests are published fully in earlier papers, (5), (6) and (8). The control animals were still sexually immature.

Animal 1 was nearly killed by the severity of the injections which were much too large for such a small animal.

A second series of injections of liquor folliculi, $\frac{1}{2}$ cc. per dose, was made into four litter sisters 24 (± 1) days old (table 2).

On the third day ($51\frac{1}{2}$ hours) after the first injection the open vagina and oestrous smear indicated positive results in the three animals injected. The condition of the control animal continued unchanged.

A third series of injections was made into normal litter sisters 35 days old, to test the effect upon general body growth (table 3). The weights of these rats ranged from 47 to 52.5 grams, average 49 grams.

Doses of 0.7 cc. of an alcoholic extract were given at first. When these were exhausted 1 cc. doses of liquor folliculi fresh daily were substituted.

At 5:30 p.m. on the 6th, although there was considerable reddening of the vulva, the opening of the vagina had not occurred. At 9:30 a.m. on the following day an open vagina was observed and oestrous smear obtained from each of the injected animals. The control animal remained in an immature condition. Nos. 12 and 12-2N were killed for histological examination. Injections were continued into 12NL and 12NR for 2 and 3 days respectively.

The control animal, 12-2N, increased 5 grams in weight in three days. Animal 12 gained 4 grams in the three days during which she was injected; 12NL, 5.5 grams in 4+ days; and 12NR, 5.5 grams in 5+ days. From these data it is obvious that injections of the hormone did not greatly retard gain in body weight.

A fourth series of injections was made into normal animals to furnish a more complete histological check on the conditions of the genital organs (table 4). Three litters, 29, 25 and 25 days of age respectively, were chosen, one animal from each being kept as a control. An open vagina was noted and an oestrous smear obtained from each injected animal. The controls remained unchanged.

So far our injections into immature animals had been made without previously removing their ovaries, depending upon litter sisters as controls. At this point the realization came that we had not proved the follicular hormone a substitute for the ovary, for conceivably something which might not actually substitute might activate the immature ovaries to produce the result.

Consequently a fifth series of injections was made into animals from which both ovaries had been removed one and two days before the first

TABLE 2

Injections of liquor folliculi into normal immature rats of the same liter, 24 (±1) days old

ANIMAL	INJECTIONS					RESULTS
	5/2			5/3		5/4
	8:30 a.m.	12:40 p.m.	5 p.m.	9 a.m.	1 p.m.	12 m. Open vagina, oestrous smear
5	½ cc.	½	½	½	½	+
5-NL	½ cc.	½	½	½	½	+
5-NR	½ cc.	½	½	½	½	+
5-2N	Control	/	/	/	/	−

TABLE 3

The effect of continued injections of the follicular hormone on immature females; 4 litter sisters 35 days of age at the beginning of the experiment

Injections: On 4/4 and 4/5, extract 22, on 4/6, extract 23,* and thereafter liquor folliculi fresh daily.

Animals		12	12-NL	12-NR	12-2N
Weight in grams, 4/4/23..		52.5	48.0	49.5	47.0
Injections					
4/4	3 p.m.	0.7 cc.	0.7 cc.	0.7 cc.	Control
	8:35 p.m.	0.7 cc.	0.7 cc.	0.7 cc.	No injections
4/5	12:45 a.m.	0.7 cc.	0.7 cc.	0.7 cc.	
	6:50 a.m.	0.7 cc.	0.7 cc.	0.7 cc.	
	11:45 a.m.	0.7 cc.	0.7 cc.	0.7 cc.	Vagina closed
4/6	12 m.	1	1	1	
	5:30 p.m.	1	1	1	
		Vagina open—oestrous smear			
4/7	9:30 a.m.	KO 56.5 grams	1	1	KO 52 grams
	4:35 p.m.		1	1	
4/8	9:50 p.m.		1	1	
	10 p.m.		KO 53.5 grams	1	
4/9	10 a.m.			1	
	5 p.m.			KO 55 grams	
Increase in weight		4 grams 3 days	5.5 grams 4+ days	5.5 grams 5+ days	5 grams 3− days

* Extracts 22 and 23 were alcohol soluble substance from follicular contents: 22 gave a positive test in a spayed adult rat in 1:20 dilution.

injection (table 5). The controls were not spayed. An open vagina was noted and an oestrous smear obtained in each of the injected animals on the third day (62 hours) after the first of five ¾ cc. injections. The controls remained unchanged.

TABLE 4

A fourth series of injections of a concentrated extract of liquor folliculi into sisters of 3 different litters

ANIMAL	AGE	6/8			6/9			6/10		6/11		6/12
		9 a.m.	12 p.m.	6 p.m.	8 a.m.	5 p.m.	10 p.m.	9 a.m.	5 p.m.	1 p.m.	5 p.m.	6 p.m.
	days											
A12	29	Control	.....	.....	.....	.....	.....	.....	KO			
A12-NL	29	½ cc.	½	½	½	½			KO			
A12-NR	29	½ cc.	½	½	½	½		½	½	¾	¾	KO
A13	25	Control	.....	.....	.....	.....	KO					
A13-NL	25	Control	.....	.....	.....	.....	.....	.....	.....	.....	.....	KO
A13-NR	25	½ cc.	½	½	½		KO					
A13-2N	25	½ cc.	½	½	½			¾		¾	¾	KO
A14	25	Control	.....	.....	.....	.....	.....	.....	KO			
A14-NL	25	½ cc.	½	½	½	½	.....	.....	KO			

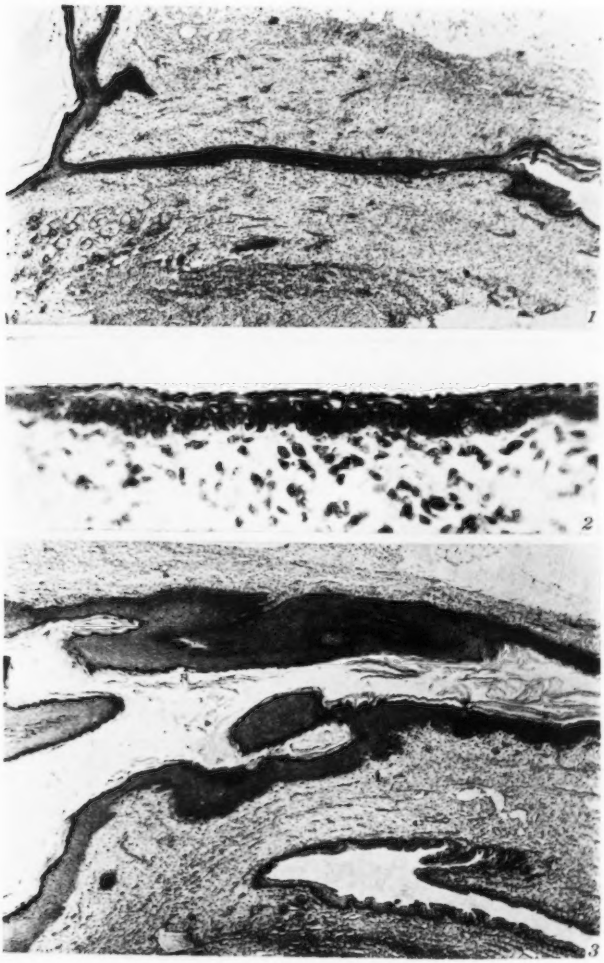
TABLE 5

A fifth series of injections into immature animals from which both ovaries had been removed one and two days before the first injection. The controls were not spayed

ANIMAL	AGE	DATE SPAYED	MATERIAL INJECTED	INJECTIONS					RESULTS
				7/26		7/27		7/28	Open vagina, oestrus smear, 11 p.m.
				9 a.m.	2 p.m.	9 a.m.	9 p.m.	9 a.m.	
	days								
15	26 (±2)		Control	.....	.....	.....	.....	.....	−
15-NL	26	7/24	L.F.	¾ cc.	¾	¾	¾	¾	+
15-NR	26	7/24	L.F.	¾ cc.	¾	¾	¾	¾	+
17	30 (±1)		Control	.....	.....	.....	.....	.....	−
17-NL	30	7/25	L.F.	¾ cc.	¾	¾	¾	¾	+

Observations upon opening the body cavities showed the uterine cornua of the control animals to be infantile. Those of the injected animals were hyperemic and considerably distended.

A SUMMARY OF RESULTS OF HISTOLOGICAL EXAMINATION: *The control animal.* A sagittal section of the vagina of control animal A-12 (see fig. 1 and table 4) represents the condition of this organ in the immature



Compare figure 1 with figures 3 and 4
Compare figure 2 with figures 5 and 6

Fig. 1. Control: outer $\frac{1}{3}$ of vagina (sagittal section). A solid cord of epithelium 4 to 8 cells thick. Rat A12, 31 days old.

Fig. 2. Control: wall of inner $\frac{2}{3}$ of vagina as seen at right of figure 1 (sagittal section). Epithelium 2 to 3 layers thick. Rat A12.

Fig. 3. Injected: outer $\frac{1}{3}$ of vagina (sagittal section). Rapid growth induced by the follicular hormone has greatly thickened the vaginal wall and caused the formation of a heavy cornified layer. This has resulted in the opening of the vaginal orifice. Rat A12-NL, 31 days old at the end of a series of injections (table 4).

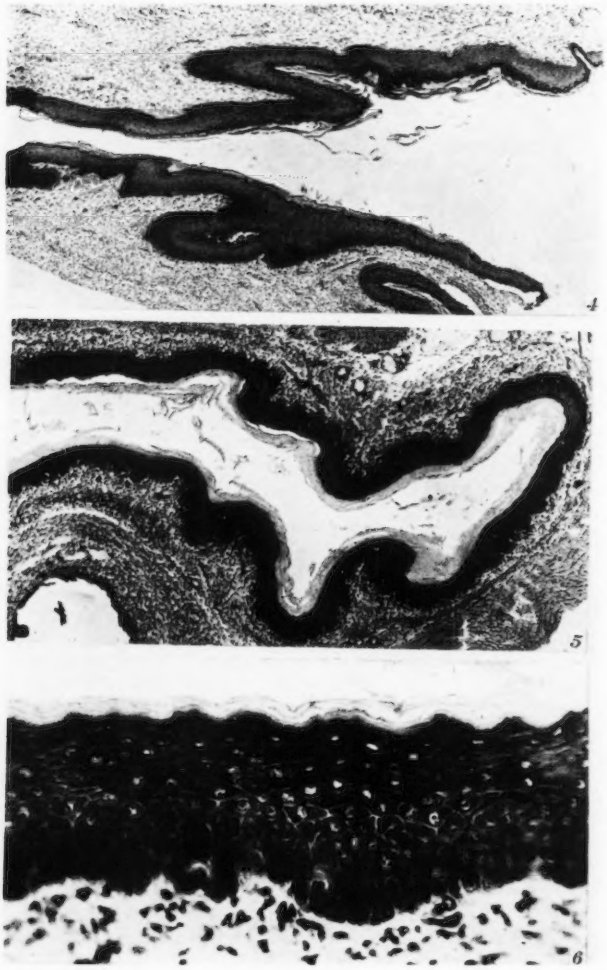
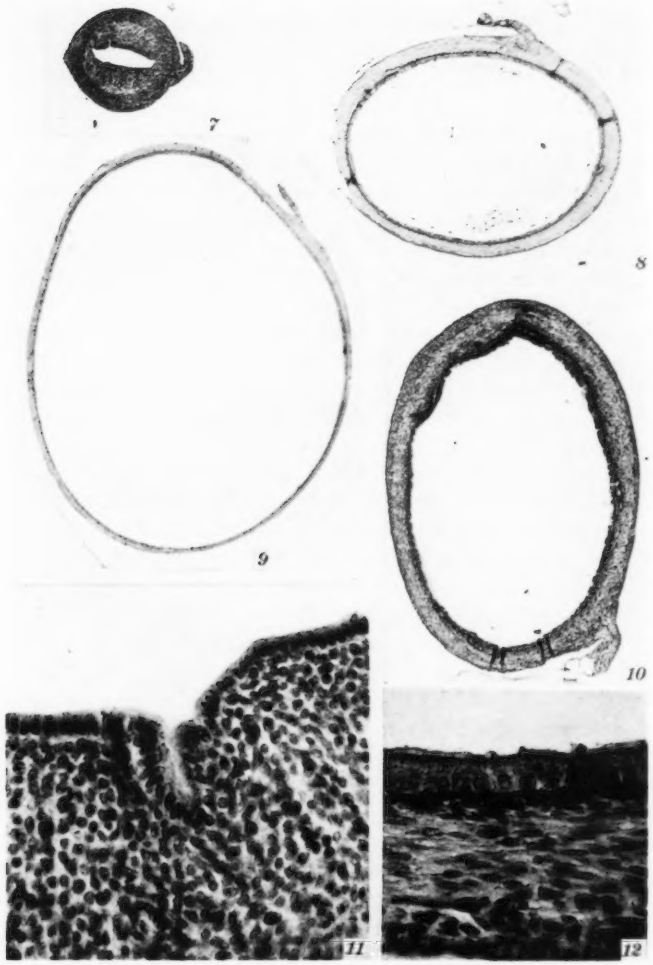


Fig. 4. Injected: outer $\frac{1}{3}$ of vagina (sagittal section). Similar to figure 3; Rat A12-NR, 33 days old (table 4).

Fig. 5. Injected: inner $\frac{2}{3}$ of vagina (cross section). Thick walled, with heavy cornified layer intact; a typical oestrous condition. Rat A12-NL, 31 days old.

Fig. 6. Injected: a higher magnification of section in figure 5; mitoses are abundant in the germinativum.



Compare figure 7 with figures 8, 9 and 10
Compare figure 11 with figure 12

Fig. 7. Control: uterus (cross section) infantile, anemic, walls collapsed. Rat A12.

Fig. 8. Injected: uterus (cross section) hyperemic, distended with secretion. Rat A12-NL.

Fig. 9. Injected: uterus (cross section) extreme degree of distention. Rat A12-NR.

Fig. 10. Injected: uterus (cross section) hyperemic, distended. Rat 12NL.

Fig. 11. Control: uterus. A higher magnification of section shown in figure 7. Epithelium and stroma undifferentiated.

Fig. 12. Injected: uterus. A higher magnification of section shown in figure 8. Typical columnar secreting epithelium and spindle shaped stroma cells.

rat. A solid cord of epithelium 4 to 8 cells in thickness constitutes the outer one-third of the vagina. The inner two-thirds have a well formed lumen shown at the right in figure 1. The walls of this region are 2 to 3 cell layers thick (fig. 2). An occasional mitotic figure (slightly to the left of the center in the basal layer) indicates that some growth is taking place.

The uterus is small and anemic (fig. 7). A few simple tubular glands have begun development. The uterine epithelium is quite undifferentiated and the stroma almost embryonic in structure (fig. 11).

The ovaries contain a large number of normal follicles at different stages of development, some in comparatively advanced stages of growth and containing considerable amounts of liquor folliculi. Several atretic follicles containing maturation spindles were also present. No corpora lutea had as yet formed.

The test animals. Sagittal sections through the vagina of animals A12-NL and A12-NR (see figs. 3 and 4, also table 4) show the typical open vagina of the test animals at its first artificially induced oestrus (compare with fig. 1). Figures 5 and 6 are cross sections of the inner one-half of the vagina at higher magnifications (compare with fig. 2). These compare favorably with conditions in the normal adult animal ((8), figs. 9 and 10) and with the oestrous condition induced in mature spayed animals by injections of the follicular hormone ((6 b), figs. 10, 11 and 12).

The vaginal wall is thick (8 to 12 nucleated cell layers) and has a well-developed superficial stratum corneum which contributes the cells of the oestrous smear, thus making it possible to follow the course of the experiment in the living animal (5). The germinal epithelium contains an exceptionally large number of mitotic figures indicative of the rapid growth which has added so many layers of cells in such a short interval. This whole epithelium including the cornified layer is a new growth caused by the injected hormone, for the former vaginal wall of the control animal represented in figure 2 has been sloughed into the lumen (8).

The condition of the uterus of injected animals is shown in figures 8, 9, 10 and 12 (compare with figs. 7 and 11). The cornua are hyperemic and greatly distended with uterine secretion. Mitoses are abundant in the epithelium which is typically columnar with the nuclei ranged along the basal membrane. The stroma cells are spindle shaped. In 48 to 60 hours the differentiation represented in figure 12 has been induced in the infantile uterus by injections of the follicular hormone (compare figs. 11 and 12).

A consideration of the effect of the injections upon the ovaries will be undertaken in a later paper.

CONSIDERATION OF RESULTS. The demonstration that pubertal development of the female genital tract depends upon the follicular hormone,

rather than upon the interstitial "puberty gland," establishes the hormonal function of the ovogenic tissue of the immature ovary. Is this hormone also responsible for the development of the secondary sex characters? This possibility has led us to a more careful study of the immature ovary with the object of comparing ovogenic and interstitial tissue. The latter is either absent or present in very small amounts in the ovaries of rats.

On the other hand, follicles in all but late stages of development are present, and some of the larger of these already contain considerable amounts of liquor folliculi. Maturation spindles are also to be found in the ova of some of the larger follicles, suggesting that they may go through partial maturation even though they are not ovulated. The periodic formation of new ova in the adult ovary (4), (10) makes it unnecessary to consider that ova in the immature ovary lie dormant until they either degenerate or are ovulated. From these points it seems probable that follicles in the immature ovary are growing and producing this hormone in small amounts but that some restraining influence inhibits their complete growth and function until just before the attainment of puberty.[1]

The results of experiments by Long and Evans are of interest in this connection. Successful maturation of ova and ovulation were induced in rats by transplanting immature ovaries to mature hosts. This may be interpreted as indicating that it is not only the actual age of the ova but also the environmental influences in the mature body which determine the time of maturation of the first ova and the consequent attainment of puberty.

From these considerations it seems probable that the development of the secondary sex characters (in this species at least) is dependent upon the follicular hormone.

SUMMARY AND CONCLUSIONS

1. The injection of the ovarian follicular hormone into immature rats (both normal and spayed) induces a sexually mature condition in the genital tract similar to that of an animal experiencing its first oestrus.

2. This may be effected in 2 or 3 days by 4 to 6 injections of an active extract as early as 26 days of age, or 20 to 50 days before the usual time of the attainment of puberty.

[1] It is suggested tentatively that *a*, the development of the follicles and consequently the secretion of the follicular hormone in the immature ovary is restrained by the greater demands of rapid pre-pubertal body growth upon available nutriment, necessary hormones, etc.; *b*, that these demands become less exacting when the growth increment curve flattens out as the animal approaches the size limit of the species, whereupon this material is diverted into reproductive channels.

3. Therefore the follicular hormone (not one from ovarian interstitial tissue) is responsible for the attainment of sexual maturity in the female.

4. From *a*, the inconstancy of typical interstitial tissue in immature ovaries of several species, and *b*, the parallelism of development of the secondary sexual characters and the genital organs, it seems probable that the secondary sex characters may also be dependent upon the follicular hormone.

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INTERSTITIAL CELL HYPERTROPHY

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In a paper, "Vasectomy on rats and guinea pigs," (23), changes in the germinal epithelium were discussed. It was noted in that study (p. 434) that very little hypertrophy of interstitial cells took place. It is here intended to discuss certain phases of the question of intertubular changes, more especially interstitial cell hypertrophy found in that material.

Interstitial cells are commonly defined as those cells lying in the space between the sex cords or seminiferous tubules which have a large, clear, vesicular nucleus with scanty, peripherally arranged chromatin. They have an abundance of cytoplasm in which is found an accumulation of lipoid material. They are not always distinguishable from the intertubular connective tissue cells from which they seem to be derived. The number of interstitial cells present varies in animals of different species and in animals of the same species. In some animals they are reported absent. By compensatory hypertrophy is meant an increase in interstitial cell mass, counterbalancing or making up for any decrease from the amount of these cells normally present.

In these experiments interstitial cell hypertrophy has followed only in those testes where degeneration of germinal epithelium took place. In many experiments where the germinal epithelium did undergo degeneration, the intertubular spaces were much increased without any hypertrophy. In such cases the increased intertubular spaces are filled with lymph. A tension and pressure hypothesis is offered to account for the hypertrophy found. No compensatory hypertrophy was anywhere seen.

For details of procedures and technique, see the paper above cited. Rats ranging in age from thirty days to senescent animals and guinea pigs ranging in age from twelve days to adults, all of good breeding stock, were used. Observations were from fourteen days to ten months following the operations. Only a few representative experiments will be given in this paper.

Experimental data: In all testes retained in the abdominal cavity, degeneration of germinal epithelium took place. In some of these testes, hypertrophy of interstitial cells was found. Two examples follow:

Rat 43–56. The right testis was fastened in the abdominal cavity fifteen days. The germinal epithelium was then found to have undergone almost complete degeneration. There was some hypertrophy of interstitial cell tissue.

Rat 54–28. Both testes were fastened, uninjured, in the abdominal cavity. When the animal was sacrificed four months later, the left testis had returned to the scrotum. Its tubular tissue was recovering from degeneration and there was some interstitial cell hypertrophy. The

TABLE 1

Experiments in which interstitial cell hypertrophy took place

ANIMAL	AGE	DURATION OF OBSERVATION
Rat 51	Senile	4 months
Rat 54	Adult	4 months
Rat 43	Adult	15 days
Rat 45	Adult	15 days

TABLE 2

Experiments in which no hypertrophy took place though the parenchyma had undergone almost complete degeneration

ANIMAL	AGE	DURATION OF OBSERVATION
Rat 36	Adult	6 months
Rat 44	Adult	2 months
Rat 46	Adult	2 months
Rat 38	Adult	1 month
Rat 28	4 months	14 days
Guinea pig 4	12 days	10 months
Guinea pig 9	30 days	10 months
Guinea pig 17	30 days	6 months
Guinea pig 32	4 months	2 months
Guinea pig 31	4 months	1 month

right testis, still in the abdominal cavity, was almost devoid of germinal epithelium and its interstitial tissue markedly hypertrophied.

Such experiments are typical of those found in the literature and more of them need not be given. From such experiments it appears that hypertrophy of interstitial tissue accompanies degeneration of seminiferous or parenchyma tissue. No case of hypertrophy of interstitial tissue was found in the absence of tubular degeration and simple vasectomy never produced it.

Degeneration of germinal epithelium is not always accompanied by interstitial cell hypertrophy. For example, in table 2 are given ten

experiments in which there was found extensive degeneration of germinal epithelium with no interstitial cell hypertrophy. It is therefore evident that such hypertrophy is not a necessary accompaniment of parenchyma

TABLE 3

Partial castration series

RAT NUMBER	AGE	DURATION	RESULTS
55 ♯ 25	Adult	2 months	Fragment attached to abdominal wall. Leucocytosis; degeneration of tubular tissue and hypertrophy of intertubular tissue
56 ♯ 26	4 months	1 month	Fragment in abdominal cavity; undergoing absorption. Tubular tissue degenerated and intertubular tissue hypertrophied
57 ♯ 46	8 months	3 months	Fragment in abdominal cavity undergoing absorption
58 ♯ 47	8 months	2 months	Fragment in abdominal cavity. Almost complete degeneration of tubular tissue and hypertrophy of intertubular tissue
59 ♯ 48	8 months	4 months	Fragment in abdominal cavity undergoing absorption
GUINEA PIG NUMBER			
42	2 months	4 months	Fragment in abdominal cavity. Tubular tissue almost degenerated, intertubular tissue hypertrophied
43	2 months	7 months	Fragment completely absorbed
44	2 months	8 months	Fragment in abdominal cavity. Tubular tissue almost completely degenerated, intertubular tissue hypertrophied
46	2 months	9 months	What appeared to be a normal testis was found in abdominal cavity. It proved to be tunica albuginea filled with fat
45	2 months	9 months	Fragment in abdominal cavity. Tubular tissue almost completely degenerated, intertubular tissue hypertrophied
47 ♯ 41	2 months	3 months	Fragment in scrotum with vas deferens occluded. Tubular and intertubular tissue appeared normal

degeneration. In every case, however, there is an increase in intertubular space filled either by interstitial cells or by lymph.

Though there may be a minimum time required for hypertrophy to take place, time does not appear to have been an important factor in the above

experiments. This is shown by a comparison of experiments in table 1, where interstitial cell hypertrophy took place in fifteen days, with experiments in table 2, where no hypertrophy took place in ten months.

In table 3 (partial castration experiments) hypertrophy is found accompanying atrophy of seminiferous tubules. In these experiments the testes were not only injured by cutting, but they were also retained in the abdominal cavity. No parenchyma degeneration took place in the testis of guinea pig 47–41 and no hypertrophy of interstitial cells was found. The results found in this group of experiments resemble those found in artificial crytorchidism, i.e., when there is degeneration of germinal epithelium, the intertubular space is increased. This intertubular space is here filled with interstitial tissue.

DISCUSSION

The experimental evidence points to the fact that interstitial hypertrophy occurs in relationship with increase in intertubular space, resulting from atrophy of the seminiferous tissue. Such hypertrophy has been found fifteen days after the operation, but in some experiments it has not been found ten months later, even when the germinal epithelium had undergone complete degeneration.

Scheunig (31) reported that interstitial cells increased to a maximum in fetuses of four months of age, decreasing suddenly with an increase in germ cells. Kasai (13) found interstitial cells abundant in four or five-months-old fetuses, decreasing at birth. One year after birth their number is very small and at puberty they are fewer. When senility comes on they increase in number, as they also do in certain diseases. This latter phenomenon was noted by Mathiew, (21) as a consequence of fever, and more recently by Mills (22) in a report on influenza deaths, and by Kostich (14), following degeneration of germinal tissue from excessive use of alcohol. Tandler and Grosz (33) found an interstitial increase with a decrease in spermatogenesis in seasonal changes of mole testes. Rasmussen (25) reported that interstitial cells of ground hogs increase in March to a maximum number in April, when spermatogenesis has ceased and seminal tubules have but a single layer of cells present. Steinach (32), Sand (28), Lipschutz (18) and Tiedje (34), following Ancel and Bouin (2), all reported degeneration of germinal epithelium and interstitial cell hypertrophy as a consequence of vasectomy. Griffiths (9) reported hypertrophy from cryptorchidism and other observers have since reported similar findings. Branca (6) found interstitial cells highly developed in testes of monkeys where only a single row of Sertoli cells remained in the seminiferous tubules. Whitehead (36) found that interstitial cells atrophied when the tubules grew in size. Humphrey (12) found that

in urodeles, interstitial cell hypertrophy follows decrease in lobular content and that these cells then atrophy with the oncoming of spermatogenesis. All changes in size of the spermatic lobules is counterbalanced by an increase or decrease in interstitial cell volume. Parhon (24) found that in drakes interstitial cells become abundant when spermatogenesis diminishes and Champy (7) reported interstitial cells varying inversely with spermatogenesis. It is important to note that in each of the above reports, interstitial cell hypertrophy has been associated with atrophy of the seminiferous tubules. The evidence conclusively points toward a cause and effect relationship between size of these tubules and volume of interstitial cells.

It seems to be an accepted fact that interstitial cells are of connective tissue origin and as Rasmussen (25) and Saintmont (27) have each said, they probably return to that state. Sand (29) expressed the idea that they are produced by a rapid transformation of connective tissue cells, their abundance being dependent upon the degree of tubular degeneration. Whitehead offered a theory of mechanical stress or pressure to account for their decrease when the tubules enlarged. The results obtained in experiments herewith reported support and offer a basis for the extension of this hypothesis. In figure 1, it will be seen that the intertubular space is greatly enlarged following atrophy of tubular content. This always takes place when already distended or filled tubules atrophy. In some cases this space is filled with lymph as in figure 1, while in other experiments, figure 2, it is filled with interstitial cells. Both types have been abundantly referred to in the literature. The first type of testis is usually described as soft and filled with a watery fluid. When these testes are sectioned, there are found large intertubular spaces described as shrinkage. If Allen's (1) method of fixation is used and care is taken not to run too much saline solution through as a washing fluid these spaces will be found full of lymph and should not be described as artifacts from shrinkage. Such spaces, filled with lymph, are favorable for multiplication and growth of connective tissue cells. These cells quickly respond, in some cases, as noted by Sand, becoming transformed into interstitial cells. In testicular grafts, unless they are inclosed in a connective tissue capsule, the intertubular spaces become filled with infiltrating connective tissue cells. Degeneration does not overcome the entire testis at one time, but some tubules may be normal while neighboring tubules are degenerated and their intertubular spaces very much enlarged. All intertubular tissue, excepting blood vessels, very often appears like interstitial cells and should perhaps be described as such. This has been seen by the writer in many sections of testes that have large intertubular spaces filled with lymph. Reinke's (26) report that interstitial cells contain crystal-like bodies which stain intensely as does the lymph, is here of special interest. With degeneration

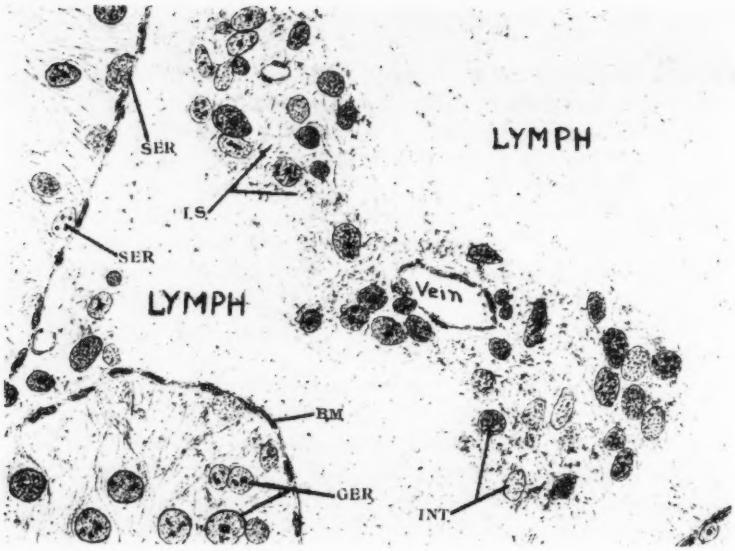

Fig. 1. Camera lucida drawing of portion of right testis of rat 51-23. *BM*, basement membrane; *GER*, germ cell; *INT*, interstitial cell; *IS*, interstitial syncytium; *SER*, Sertoli cell. Note that all intertubular cells are here interstitial cells, not connective tissue cells.

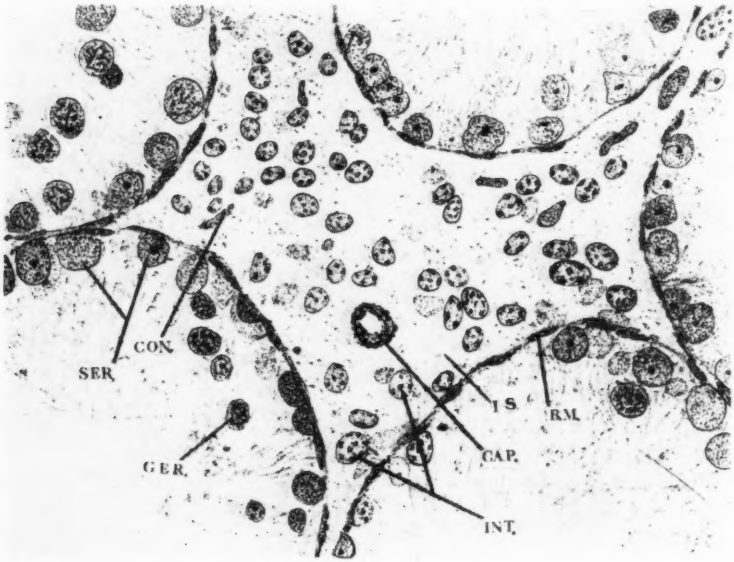

Fig. 2. Portion of testis of guinea pig 41. *CAP*, capillary; *CON*, connective tissue cell. The interstitial tissue here fills all the intertubular space.

of germinal epithelium, the seminiferous tubules decrease in size, thereby increasing the intertubular spaces. These spaces fill with lymph, bathing the connective tissue found therein and supplying it with abundant space and food for growth and proliferation. That this is what happens, is shown by the fact that cells intergrading in structure between connective tissue cells and interstitial cells are frequently found. The additional fact that the interstitial cells stain like the lymph in which they are bathed further supports this hypothesis. If the tubules again become active and enlarge, the pressure they exert upon these spongy, lipoid-filled cells causes them to decrease in volume or to atrophy, as was noted by Whitehead. This mechanistic hypothesis seems far more workable than such teleological theories that ascribe to the interstitial cells a definite trophic function of storing up food for a possible future use of the germinal tissue, should it regenerate.

There are a few reports in the literature where no interstitial hypertrophy has followed degeneration of parenchyma tissue. Barrott and Arnold (5) found no interstitial cell changes two weeks after x-ray treatment, though the tubular tissue had suffered degeneration. Villimin's (35) findings were similar and Hooker (11) found no changes of interstitial cells following the same procedure. Had these tissues been prepared by Allen's method of fixation with special care as mentioned above, it is very probable that the intertubular spaces would have been found very much enlarged and filled with lymph as in figure 1. Gould (8) found no hypertrophy in an hermaphroditic pig testis, though there was no spermatogenesis and the tubules were only about two-thirds normal size. The testis was much smaller than a normal pig testis. In such cases, the testis never has developed and there is no true degeneration. The small size of the testis itself has in a way counterbalanced the smallness of the tubules. Many testes of the present series are smaller than normal, though none are small enough to completely counterbalance the decrease in tubular size. These two types of findings are not discordant to the hypothesis here given. There are, however, a few reports that do not seem in accord with this hypothesis. For example, Marshall (20) reported that the interstitial cells increased in number simultaneously with spermatogenesis. At such times the testis, however, increases enormously in size.

Compensatory hypertrophy: Ancel and Bouin (3) stated that by removing one testicle of a rabbit and ligating the vas deferens of the contralateral testis for a period of six months, they secured degeneration of the seminal gland and the interstitial gland became twice the normal size. From this they concluded that the degeneration was produced by the ligation and the hypertrophy by the removal of the other testicle. In another paper (4) they state that the interstitial cells alone submit to a compensatory

hypertrophy in unilateral cryptorchid testes, attaining a weight double that of the contralateral ectopic testes. Hanes (10) reported compensatory hypertrophy of interstitial cells in mono-cryptorchid pig testes where the contralateral testes were removed, stating, "The hypertrophy is much more marked than when the contralateral testis is allowed to remain as in Ancel et Bouin's experiments." Sand (30) reported that he produced compensatory hypertrophy of interstitial cells in three experiments on rabbits by ligating one vas deferens and removing the contralateral testis. Lipschutz (18) on the other hand, reported that out of four unilateral castration experiments, observed over a year, only one testicle was greater in weight than that of any normal testicle. Kyrle (17) made semi-castrations on two fully grown dogs; removing the remaining testes three and six months later. They differed in no respect from the normals. Lipschutz, Ottow and Wagner (19) did not secure compensatory hypertrophy by removing one testis and a large part of the second testis, though they found degeneration of the germinal epithelium. Kropman (15) found no epithelial degeneration and no interstitial cell hypertrophy in the same type of experiments. Kuntz (16) secured no hypertrophy or hyperplasia in unilateral castration experiments upon rabbits for thirty-eight days. In his opinion, hypertrophy of interstitial tissue in cryptorchid testes is probably an accompaniment of degeneration of germinal epithelium, rather than a compensatory phenomenon.

Eight experiments touching on this point are here given. In each of four guinea pigs, no. 3, age twelve days; no. 19, age thirty days; no. 35, age six months and no. 26, age four months, one testis was removed with no injury to the contralateral testis. At ten, six, six and two months later, respectively, the animals were sacrificed and the testes all found normal. From each of guinea pigs no. 7, age twelve eays; no. 14, age thirty days; no. 36, age six months and no. 28, age four months, one testis was removed and the vas deferens of the contralateral testis ligated. At ten, six, six and two months later, respectively, the animals were killed and the remaining testis studied. In each case it was normal. Age seems to be of little importance since animals of twelve days to six months yield the same results. Likewise, time does not seem to be an important factor since experiments of two to ten months' duration all yield like results. Semi-castration and semi-castration plus ligation of the vas deferens of the remaining testes did not cause any hypertrophy of interstitial cells in the above experiments. Only where there is found degeneration of germinal epithelium is there found any hypertrophy of interstitial cells. On the other hand, both testes may undergo extensive degeneration with accompanying interstitial cell hypertrophy and thereby increase their number many times. This increase cannot be a compensatory hypertrophy. It is only an adjustment of connective tissue cells to an increase of intertubular

space filled with lymph. The term compensatory seems applicable only in the sense of making up for or counterbalancing an increase of intertubular space caused by decrease in the size of the seminiferous tubules.

It seems logical to conclude from a consideration of the material in hand and from a consideration of the literature that:

1. Interstitial cell hypertrophy is found associated with atrophy of germinal epithelium.

2. Atrophy of germinal epithelium is not always accompanied by interstitial hypertrophy.

3. In cases where there is degeneration of germinal epithelium with no interstitial cell hypertrophy, the intertubular spaces are increased in size and filled with lymph.

4. Only a very short time is required for the development of interstitial cell hypertrophy and neither this nor age has been a factor in the above experiments or in the discrepancies in the literature.

5. "Partial castration" experiments yield results similar to those of cryptorchidism.

6. Compensatory hypertrophy, in the sense defined above, does not take place.

7. Tension and pressure are important factors in the production of an increased number of interstitial cells.

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STUDIES IN FATIGUE

XV. Further Studies on the Staircase Phenomenon in Mammalian Skeletal Muscle

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In a recent communication (1) it was shown that the "Treppe" phenomenon in cat's skeletal muscle was not due to a prolongation of the contraction phase as Fröhlich (2) and Adrian (3) thought to be the case in frog's skeletal muscle. Although no evidence was given then to show whether the inertia of the lever had any influence on the staircase phenomenon, it was felt to have little or none. Fortunately, since then it has been possible to clear up this question by repeating the work with some modifications and also with a frictionless lever.

Method. Cats were used in these experiments. They were anesthetized either with ether by inhalation or with urethane 2 grams per kilo by stomach. In some experiments the apparatus and the method were the same as those employed in the previous work (1) except in one particular. After the control readings were made and the muscular contraction was about three-fourths developed it was abruptly limited but the lever allowed to overshoot if it would. In this manner the degree of fling of the lever was recorded.

In the later experiments the muscle contracted against a frictionless lever similar to that devised by Hill and Hartree (4). Instead of a hack saw blade a piece of a clock spring 40 cm. long and 1.9 cm. wide was used. It was soldered to the ends of a heavy U-shaped iron bar the ends of which when free sprung apart 5 mm. To the center of the spring was soldered a brass bar 1 mm. by 2 mm. by 6.9 cm. so that one end extended 1.5 cm. and the other 3.5 cm. beyong the width of the spring. A small mirror 3 mm. square was glued to the shorter end of the rod and the muscle was attached to the longer one. This lever has a frequency of 1/160 second.

The contractions were recorded by the mirror reflecting rays of light from an arc-light into a photographic registering apparatus. The beams of light from the arc were focused upon the mirror by passing them through a convex lens. The reflected rays were focused upon the sensitized bromide paper in the camera by a convex cylinder. The light appeared as a vertical

line which moved horizontally in front of the horizontal slit in the photographic registering apparatus when the lever was moved. The magnification of the contractions by the apparatus was seven times. The rate of stimulation was either 120 or 220 times a minute. The time in seconds and 1/100 seconds was also recorded photographically by using small mirrors on the chronometer and electrically driven tuning fork to reflect the light into the camera.

In the early experiments with this method the sensitized paper was moved slowly back of the slot so that the contractions appeared as straight lines. This was done to determine if the "Treppe" phenomenon existed with a lever that was frictionless, of high frequency and practically flingless. In the later experiments the sensitized paper moved more rapidly to allow for the calculation of the duration of the contraction phase of the muscular contraction.

RESULTS. That the increased height of contraction in "Treppe" cannot be explained away by the inertia or fling of the writing lever can be seen in figure 1. In this animal the degree of shortening of the muscle was abruptly limited, the muscle lever, however, being allowed to rise as far as it would. Curve *1* is the first; *2*, the one hundred-fiftieth; and *3*, the one hundred-fifty-first contraction, the last with limited muscle shortening. By this method it has been possible to determine how much the lever may overshoot. In some instances it was found to overshoot as much as 8 per cent, never as much as 100 per cent, to which height of betterment the muscles frequently contract after repeated stimulation.

Photographic method. The results with the photographic method confirm the findings mechanically recorded by the lever as just given. (See figs. 2, 3, 4 and 5.) In figure 2 the height of muscular contraction increased from 20 mm. first contraction to 46 mm. one hundredth contraction, or an increase of 130 per cent. Inasmuch as the inertia of the lever was practically zero and the frequency short (1/160 second) the betterment here shown cannot be attributed to a defect in the lever. This betterment must therefore be the result of some change within the muscle itself.

The above cited experiment however does not answer the question as to whether or not the increased height of contraction is caused by a prolongation of the contraction phases as previously stated by other experimenters (2), (3) on the frog's skeletal muscle. In order to confirm or disprove my earlier conclusions (1) another series of experiments was performed in which the movements of the high frequency lever were photographed upon rapidly moving sensitized paper and the duration of the contraction period calculated. Figures 3, 4 and 5 are such photographs. The photographs seen in figures 3 and 4 were made from the same muscle. A comparison of these records illustrates a marked difference in the height of the "Treppe" phenomenon. This is much more marked in figure 4. These

results, which are constant for cats' skeletal muscles, illustrate quite definitely a difference between the muscles of homothermic and poikilothermic animals. It is rarely possible to get the staircase effect in the muscles of poikilothermic animals after they have been partially fatigued. With the muscles of homothermic animals this effect comes to the foreground after the muscle has been partially fatigued and allowed to rest

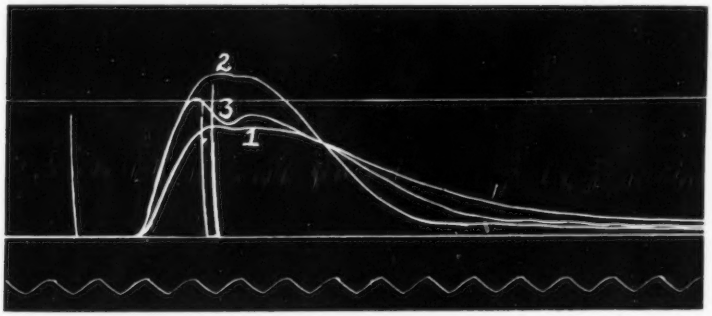

Fig. 1. Cat. Ether anesthesia. Isometric contractions of anterior tibial muscle. The steel spring had an initial tension of 70 grams with an increase of 7 grams for each centimeter the writing point moved vertically on the drum's surface; the anterior tibial nerve (peroneus communis) was excited with a maximal break induction shock. The muscle contracted 100 times a minute. Time in 0.01 second. Magnification of the contraction by the lever is 3 times and record reads from left to right. *1*, first; *2*, one hundred fiftieth; and *3*, one-hundred-fifty-first contractions. Muscular contraction in *3* limited but lever allowed to overshoot thus showing the degree of fling of lever.

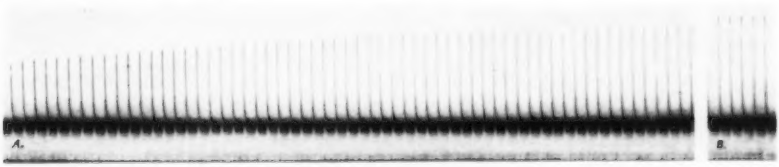

Fig. 2. Cat. Urethane anesthesia. Upper photographic record of contractions of anterior tibial muscle with frictionless lever of 1/160 second frequency. Muscle contracted 120 times a minute. Lower time in seconds. A: first to sixtieth contraction. B: one hundredth to one-hundred-fifth contraction.

for a short time before beginning the series of contractions to demonstrate the "Treppe" phenomenon.

The animal, cat, used in figures 3 and 4, weighed 3 kilos and received 6 grams of urethane by stomach tube. The anterior tibial muscle was

stimulated through its nerve 120 times a minute. In figure 3 are shown the first, tenth, fiftieth and one hundredth contractions. The periods of contraction were found to be 0.028, 0.02, 0.018 and 0.018 second respectively, while the heights of contraction were 9, 10, 11 and 13 mm. There was a maximal increase in the height of contraction of 44 per cent and a simultaneous decrease in the duration of the contraction phase of 36 per cent.

After the muscle had contracted one hundred times, as seen in figure 2, and again three hundred times with a rest of fifteen minutes between it and the first series of contractions, the third series of contractions was photographed as seen in figure 4. Before the muscle was stimulated it had rested twenty minutes. The rate of stimulation was 120 times a minute. In this figure are shown the first, tenth, twentieth, fiftieth, one hundredth, one hundred-fiftieth, two hundredth, two hundred-fiftieth, three hundredth and four hundredth contractions. The times of the contraction phases in seconds and heights of contractions in millimeter were 0.025, 1; 0.022, 2; 0.02, 2.5; 0.018, 3.5; 0.018, 7; 0.018, 8; 0.016, 9; 0.016, 9; 0.018, 9; and 0.019, 9. In this third series the maximum height of contraction was increased by activity 800 per cent, while the contraction phase was decreased in duration 36 per cent.

A cat weighing 5 kilos was used in figure 5. The anterior tibial muscle was stimulated through its nerve 220 times a minute. The first, tenth, fiftieth, one hundredth, two hundredth, three hundredth, four hundredth and five hundredth contractions are shown. The durations of the contraction period of these contractions are as follows; 0.03; 0.028; 0.022; 0.016; 0.015; 0.018; 0.02; 0.02 second while the heights of these contractions were 13, 14, 15, 21, 23, 21 and 18 mm. The maximum decrease in the contraction phase during activity was 50 per cent and the maximum increase in the height of contraction was 77 per cent.

Discussion. From these results and those previously given (1) it appears that the change in the height of muscular contraction is independent of the change in the duration of the contraction period. In the 45 readings previously made on 31 animals (1, p. 342) it will be recalled that the contraction period had decreased on the tenth contraction from 0.027 second to 0.019 while the height of muscular contraction had actually decreased from 14 to 13 mm. If we take the first series of readings on the 31 animals the height remains at 13 mm. although the same decrease occurs in the contraction phase. On the one hundredth contraction the duration of the contraction phase was 0.021 second but the height of muscular contraction is now 22 mm. and on the two hundredth contraction the duration of the contraction phase was 0.022 second and the height of muscular contraction was 25 mm. In figure 5 it will be seen that the height of contraction increased from 13 the

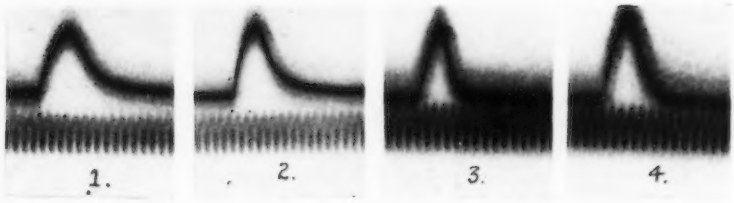

Figure 3.

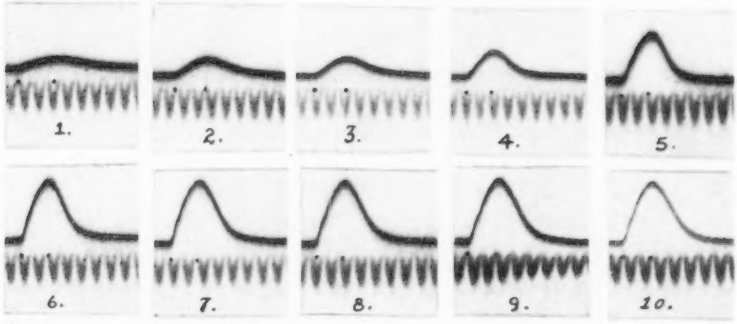

Figure 4.

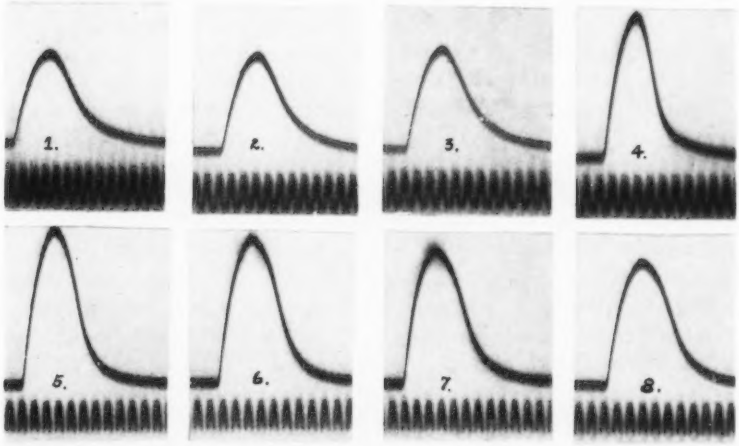

Figure 5.

Fig. 3. Cat, 3 kilos. Urethane anesthesia. In this and the following two figures the rate of stimulation was 120 times a minute. The tuning fork vibrations mark 0.01 second *1*, 1; *2*, 10; *3*, 50; and *4*, 100th contractions. In this and the following records the apparatus used was a frictionless lever with a frequency of 1/160 second.

Fig. 4. Same as figure 3, only thirty-five minutes later after rest. (The muscle having contracted three hundred times, fifteen minutes after figure 4 was made.) *1*, 1; *2*, 10; *3*, 20; *4*, 50; *5*, 100; *6*, 150; *7*, 200; *8*, 250; *9*, 300; and *10*, 400th contractions.

Fig. 5. Cat, 5 kilos. Urethane anesthesia. Anterior tibial muscle stimulated 220 times a minute through its nerve. Apparatus same as in figure 3. *1*, 1; *2*, 10; *3*, 50; *4*, 100; *5*, 200; *6*, 300; *7*, 400; and *8*, 500th contractions.

first to 23 mm. on the two hundredth contraction while simultaneously the duration of the contraction period decreased from 0.03 to 0.015 second after which the height of contraction decreased to 18 mm. on the five hundredth contraction while the duration of the contraction period lengthened to 0.02 second.

SUMMARY

1. The "Treppe" phenomenon discussed previously is not the result of the inertia of the lever employed.

2. The "Treppe" phenomenon was demonstrated photographically in cat's skeletal muscle with a frictionless high frequency (1/160 second) lever.

3. The staircase phenomenon is not dependent upon the prolongation of the contraction phase but is independent of the duration and dependent upon some change within the muscle itself.

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EFFECT OF STIMULATION OF AFFERENT NERVES UPON THE RATE OF LIBERATION OF EPINEPHRIN FROM THE ADRENALS

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In a paper published in 1916 we stated that with stimulation of the central end of the brachial nerves we obtained no increase whatever in the rate of epinephrin output (1). The observations were made on cats, adrenal vein blood being collected in a cava pocket during and without stimulation of the brachial for equal times, and then released. The rise of blood pressure caused by the blood from the pocket was the same whether the nerves had been stimulated or not.

Later we confirmed this result by collecting blood from the cava pocket in cats and dogs and then assaying it on rabbit intestine segments (2). Stimulation of afferent nerves (sciatic and brachial) produced no detectable increase in the rate of liberation of epinephrin.

The characteristic dilatation of the pupil and other reactions caused by epinephrin in an eye sensitized by previous removal of the corresponding superior cervical ganglion cannot be utilized to test the question, since dilatation is caused in both eyes through the nervous system when afferent nerves are stimulated. However, we have noted that the reactions caused by stimulation of the sciatic are distinctly different from those obtained when they are elicited by epinephrin injected into the circulation or liberated into it by stimulation of the peripheral end of the splanchnic (3).

We observed no essential difference in the constriction of the denervated limb, caused by blood pent up in the adrenals by clipping the veins and then released after a definite time, whether the central end of the contra-lateral sciatic had been stimulated or not during the period of clipping off (4).

We are therefore forced to the conclusion that reactions relied on by certain observers as proving a reflexly increased output of epinephrin (5), and easily elicited under the experimental conditions employed by us for collection of the adrenal vein blood, are not due to an increased output of epinephrin, since we could not fail to detect this, if present, by our methods.

To see whether by any chance we could ever obtain a different result from that previously obtained with the rabbit segments, we have recently repeated the work on 8 dogs and 9 cats. One experiment, on a cat, was not used because the assay was not good enough. Our conclusion is precisely the same as before. In not one of these animals, whether lightly (as in the great majority) or more deeply anesthetized (as in one or two experiments), have we ever seen any evidence of an increased output of epinephrin caused by stimulation of the central end of the median or the brachial nerves. In most of the animals the experiment was concluded by stimulation of one of the major splanchnics. We were thus in a position to compare the effect on the segments of adrenal vein blood collected while the output of epinephrin was indubitably undergoing an increase, through direct stimulation of the efferent secretory fibers, with the effect of blood collected during sensory stimulation when the same efferent fibers are alleged to be reflexly excited. The reader cannot fail to be struck with the fact that the segment test shows a great increase in the epinephrin output during splanchnic stimulation but none at all during brachial stimulation, although the ordinary signs of effective afferent excitation (increased respiratory movements, rise of blood pressure, etc.) were all present. In other words, the method detects real but not imaginary epinephrin.

Simple as the method would appear to be in principle it seems to be still possible for writers (6), claiming to have followed it "faithfully," to publish papers demonstrating that they do not understand how to apply it. We therefore feel obliged still to reproduce a fair number of tracings. And while it is practically impossible to publish all the tracings belonging even to one complete assay, we shall reproduce from two of the experiments (dogs 981 and 985) a sufficient number to indicate how the concentrations of epinephrin in the various specimens are arrived at. Very few tracings need then be given from the rest of the experiments. We must again point out that in approximating to the concentration of a specimen it is generally far more important to fix clearly an upper and a lower limit than to seek at once for an exact match. Another point is worthy of attention; it is a great mistake to collect and to attempt the assay of a large number of blood samples in any one experiment. Unless, indeed, the investigator has enough competent assistance to enable him to run the assay simultaneously on two segments, testing a certain number of the specimens on each, it is impossible to make enough observations to assay properly 16 or 17 specimens, as a recent writer (6) alleges that he did habitually.

The dogs in our experiments were all anesthetized with ether, as were 5 of the cats. The remaining 3 cats (979, 980 and 982) received urethane by stomach tube. When samples were collected during stimulation of nerves the stimulation was begun a little before the beginning of

collection of the sample to allow for the dead space. The cava pocket was short and the cannula narrow so as to reduce the dead space as much as possible. The capacity of the cannula was only 0.1 to 0.2 cc. for cats and 0.5 cc. for dogs.

While, as more than once pointed by us, a segment often tends to improve as it is worked with and we can generally use the first segment

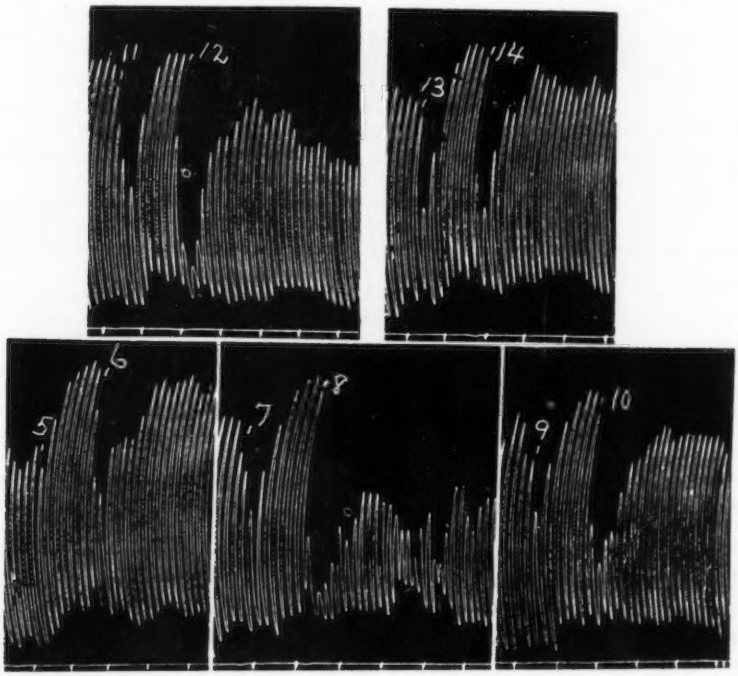

Fig. 1. Intestine tracings. Blood from dog 981. At *5*, *7*, *9*, *11* and *13* Ringer's solution was replaced by indifferent blood, and this at *6*, *8* and *12* by indifferent blood to which was added adrenalin to make up concentrations of 1:6,100,000, 1:3,660,000 and 1:4,900,000 respectively. At *10* the indifferent blood was replaced by adrenal vein blood specimen II, collected without nerve stimulation, and at *14* by specimen IV, collected during stimulation of the central end of the left median nerve. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). As in all the tracings, the time is marked in half minutes. Reduced to two-thirds.

prepared right through the assay, we never hesitate to take a second rabbit if the first seems unsatisfactory. Usually when one segment from a rabbit behaves badly for our purpose others taken from the same rabbit are also likely to be unsatisfactory. Any stock of intestine which we keep is covered with Ringer's solution in a shallow dish and kept cool.

Condensed protocol. Dog 981 (female). Weight 5.3 kgm. Ether. Cava pocket completed and cannula inserted at 9:15 a.m. Discarded more than enough blood to empty the dead space.

9:20 a.m. Adrenal vein blood specimen I collected, 3.6 grams in 30 seconds (7.2 grams per minute).

9:20½ a.m. Specimen II collected, 3.6 grams in 30 seconds (7.2 grams per minute). Mean arterial pressure 136 mm. Hg. Now placed central end of left median nerve on shielded electrodes and inserted a fresh cannula into cava pocket. Allowed adrenal vein blood to escape from the cannula for 35 seconds and discarded it.

9:25½ to 9:26 a.m. Specimen III, 3.9 grams in 30 seconds (7.8 grams per minute). Blood pressure 142 mm.

9:25¾ a.m. Began stimulation (coils 8 cm. apart).

9:26 to 9:26½ a.m. Specimen IV (with continuous stimulation), 4.35 grams in 30 seconds (8.7 grams per minute). Blood pressure 156 mm.

9:26½ to 9:27 a.m. Specimen V with off and on stimulation, 5.35 grams in 30 seconds (10.7 grams per minute). Blood pressure 160 mm.

9:27 a.m. Specimen VI without stimulation, 4.1 grams in 30 seconds (8.2 grams per minute). Blood pressure 150 mm. Now put shielded electrodes on left splanchnic below the diaphragm. Inserted a fresh cannula into cava and discarded adrenal vein blood for 30 seconds.

9:45 a.m. Collected specimen VII with continuous stimulation of the splanchnic (coils 8 cm.), 4.8 grams in 30 seconds (9.6 grams per minute). Blood pressure 170 mm.

9:45½ a.m. Specimen VIII with off and on stimulation of splanchnic, 4.5 grams in 30 seconds (9.0 grams per minute). Blood pressure 183 mm. Inserted a fresh cannula into cava and at 9:56 a.m. discarded the adrenal vein blood for 1 minute.

9:57 a.m. Specimen IX, without stimulation, 3.25 grams in 30 seconds (6.50 grams per minute). Blood pressure 120 mm.

Obtained indifferent blood from abdominal aorta. Left adrenal weighed 0.350 gram, and right 0.378 gram.

Specimens I and II, collected without stimulation of nerves, gave about the same reaction with the intestinal segment. They were decidedly stronger than 1:6,100,000 (*6* and *10*, fig. 1, and other observations not reproduced), much weaker than 1:3,660,000 (*8* and *10*, fig. 1), somewhat less than 1:4,900,000 (*10* and *12*, fig. 1). Later on in the assay it was shown that specimen II was stronger than 1:7,300,000 (tracings not reproduced), weaker than 1:4,900,000, much weaker than 1:3,660,000, and rather stronger than III (tracings not reproduced). II was found to be distinctly stronger than V (*82*, *84*, fig. 4). Specimen II was assayed at 1:5,300,000, corresponding to an output of 0.0014 mgm. per minute for the dog, or 0.00026 mgm. per kgm. per minute.

III, collected without stimulation of nerves, was decidedly weaker than 1:4,900,000 (*70*, *72*, fig. 5) and somewhat weaker than II (tracings not reproduced). It was assayed at 1:5,600,000, corresponding to an epinephrin output of 0.0014 mgm. per minute for the dog or 0.00026 mgm. per kgm. per minute, the same as II.

IV, collected during stimulation of the median nerve, was decidedly weaker than 1:4,900,000, somewhat weaker than II (*10*, *12*, *14*, fig. 1), not much different from 1:6,100,000, weaker than VI (tracings not reproduced). It was assayed at 1:5,800,000, corresponding to an output of 0.0015 mgm. per minute or 0.00028 mgm. per kgm. per minute.

V, collected during off and on stimulation of the median nerve. was somewhat weaker than 1:6,100,000 (*64*, *66*, fig. 4), weaker than II (*82*, *84*,

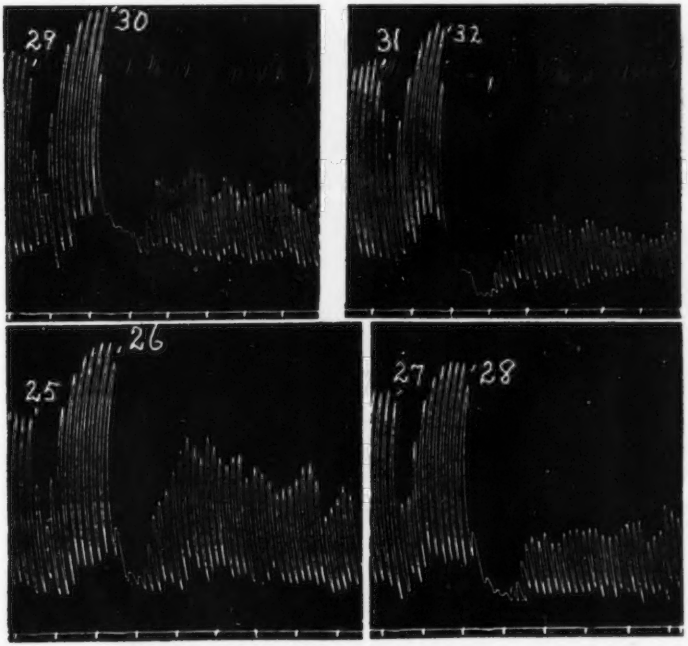

Fig. 2. Intestine tracings. Blood from dog 981. At *25*, *27*, *29* and *31* Ringer's solution was replaced by indifferent blood and this at *28* by adrenal vein blood specimen VII, collected during stimulation of the left splanchnic, and at *26*, *30* and *32* by indifferent blood to which adrenalin was added to make up concentrations of 1:2,450,000, 1:1,220,000 and 1:610,000 respectively. All the bloods were diluted with 3 volumes Ringer (the adrenal bloods after adding the adrenalin). Reduced to two-thirds.

fig. 4) very much weaker than VII, decidedly weaker than VIII or VI, and than 1:4,900,000 (tracings not reproduced). V was assayed at 1:6,500,000, representing an output of 0.0016 mgm. per minute or 0.00030 mgm. per kgm. per minute.

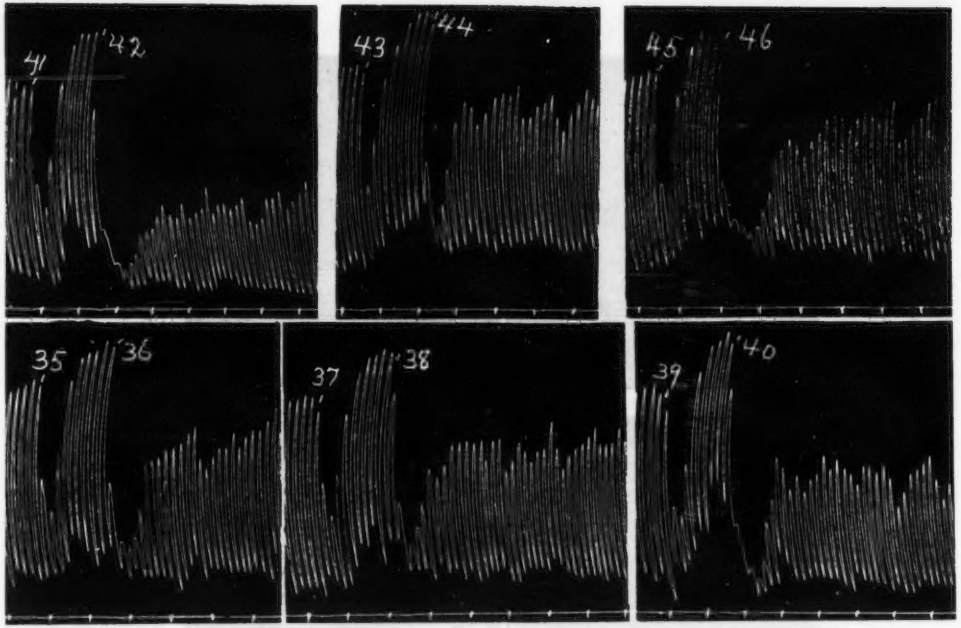

Fig. 3. Intestine tracings. Blood from dog 981. At *35*, *37*, *39*, *41*, *43* and *45* Ringer's solution was replaced by indifferent blood, and this at *36*, *38* and *46* respectively by adrenal vein blood specimens VIII (collected during splanchnic stimulation), IX (collected without stimulation of nerves) and VI, collected without stimulation of nerves. At *40*, *42* and *44* the indifferent blood was replaced by indifferent blood to which was added adrenalin to make up concentrations of 1:3,660,000, 1:3,000,000 and 1:4,900,000 respectively. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to two-thirds.

Specimen VI, collected without stimulation of nerves, was decidedly stronger than 1:4,900,000, not as strong as 1:3,660,000, much weaker than 1:3,000,000, (*40*, *42*, *44*, *46*, fig. 3), decidedly stronger than specimens IV and V and than 1:6,100,000 (tracings not reproduced). VI was assayed at 1:4,300,000, giving an output of 0.0019 mgm. per minute or 0.00036 mgm. per kgm. per minute.

Specimen VII, collected during stimulation of the splanchnic, was far stronger than any of the other specimens (tracings not reproduced), much stronger than 1:2,450,000, as strong as 1:1,220,000, decidedly

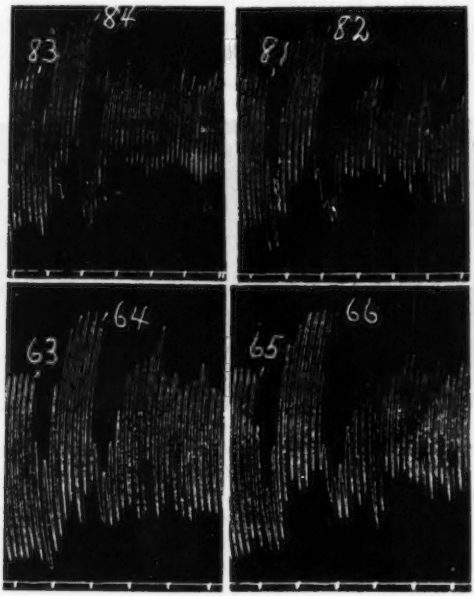

Fig. 4. Intestine tracings. Blood from dog 981. At *63*, *65*, *81* and *83* Ringer's solution was replaced by indifferent blood and this at *64* and *84* by the adrenal vein blood specimen V (collected during stimulation of the median nerve). Tracing *84* was taken much later in the assay than tracing *64*. At *82* the indifferent blood was replaced by adrenal vein blood specimen II (collected without stimulation of nerves). At *66* the indifferent blood was replaced by indifferent blood to which was added adrenalin to make up a concentration of 1:6,100,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

weaker than 1:610,000 (*26*, *28*, *30*, *32*, fig. 2). VII was assayed at 1:1,220,000, corresponding to an output of 0.0079 mgm. per minute, or 0.0015 mgm. per kgm. per minute.

VIII, collected during off and on stimulation of the splanchnic was stronger than any of the other specimens except VII, much weaker than VII, weaker than 1:3,660,000 (tracings not reproduced). VIII was not much different from IX, both somewhat weaker than 1:3,660,000, far weaker than 1:3,000,000, stronger than 1:4,900,000 (*36*, *38*, *40*, *42*, *44*, fig. 3). VIII was assayed at 1:4,000,000, giving an output of 0.0022 mgm. per minute, or 0.00041 mgm. per kgm. per minute.

IX was also taken at 1:4,000,000, giving 0.0016 mgm. per minute, or 0.00030 mgm. per kgm. per minute.

In this experiment it is obvious that while direct stimulation of one splanchnic increased the epinephrin output to 6 times the initial output, or 5 times the output before splanchnic stimulation (which would have been 10 times if both splanchnics had been stimulated), there was no

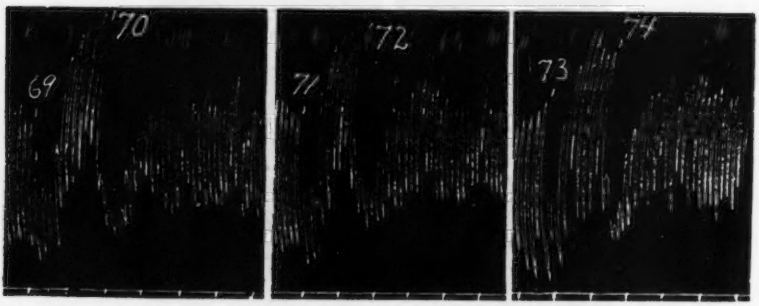

Fig. 5. Intestine tracings. Blood from dog 981. At *69*, *71* and *73* Ringer's solution was replaced by indifferent blood and this at *72* by adrenal vein blood specimen III (collected without nerve stimulation) and at *70* and *74* by indifferent blood to which was added adrenalin to make up concentrations of 1:4,900,000 and 1:7,300,000 respectively. All the bloods were diluted with 3 volumes Ringer (the adrenal in bloods after adding the adrenalin). Reduced to two-thirds.

reflex increase (within the limits of the experimental errors) when the central end of the median nerve was excited.

Condensed protocol. Dog. 985. Female. Weight 8.8 kgm. Ether. At 10:40 a.m. cava pocket completed and cannula inserted. Discarded the first blood.

10:43 a.m. Adrenal blood specimen I collected, 8.10 grams in 30 seconds (16.20 grams per minute). Blood pressure 110 mm. Hg.

10:43½ a.m. Specimen II, 9.02 grams in 30 seconds (18.04 grams per minute). Blood pressure 114 mm. Hg. Placed shielded electrodes on brachial, including median nerve. Fresh cannula inserted, and blood discarded for 1 minute.

10:59 a.m. Specimen III, 10.48 grams in 30 seconds (20.96 grams per minute). Blood pressure 110 mm.

10:59½ a.m. Specimen IV, collected during stimulation of the brachial (distance of coils 8 cm.) for 30 seconds 9.72 grams in 30 seconds (19.44 grams per minute).

11:00 a.m. Specimen V with off and on stimulation of brachial (coils 7 cm. apart), 10.17 grams in 30 seconds (20.34 grams per minute). Now placed electrodes on left splanchnic and inserted fresh cannula. Discarded blood for 1 minute.

11:10½ a.m. Specimen VI, 8.04 grams in 30 seconds (16.08 grams per minute). Blood pressure 94 mm.

11:11 a.m. Specimen VII, collected during stimulation of splanchnic (coils 7 cm. apart), 8.70 grams in 30 seconds (17.40 grams per minute).

11:11½ a.m. Specimen VIII, with off and on stimulation of splanchnic (coils 6 cm.), 12.03 grams in 30 seconds (24.06 grams per minute). Blood pressure 120 mm. Collected indifferent blood from abdominal aorta. Left adrenal weighed 0.580 gram, right 0.630 gram. At necropsy it was found that the right lumbar vein

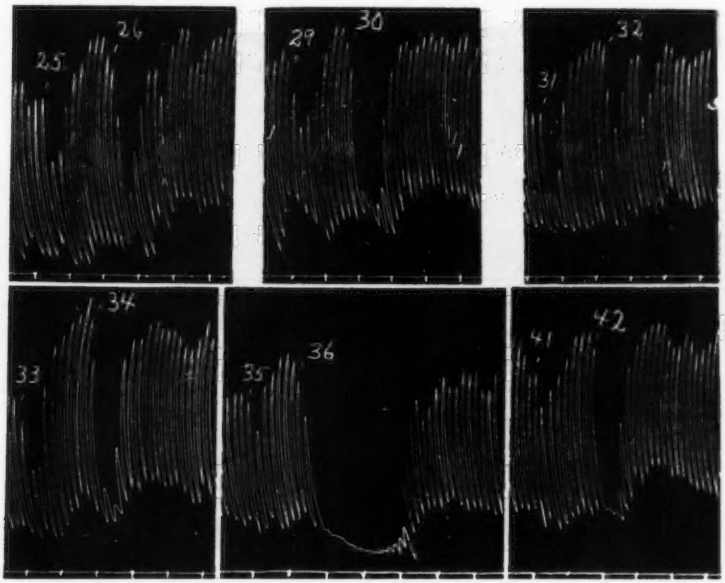

Fig. 6. Intestine tracings. Blood from dog 985. At *25*, *29*, *31*, *33*, *35* and *41* Ringer's solution was replaced by indifferent blood and this at *26* by adrenal vein blood specimen III, collected without stimulation of nerves; at *30* by specimen II, collected without stimulation of nerves; at *32* and *34* by specimens IV and V respectively, collected during stimulation of the brachial nerves; at *36* by specimen VII, collected during stimulation of the splanchnic nerve; and at *42* by specimen VI, collected without stimulation of nerves. All the bloods were diluted with 3 volumes Ringer. Reduced to three-fifths.

passed dorsal instead of ventral to the adrenal, and the adrenal vein entered the dorsal surface of the cava. For this reason the ligature only occluded one branch of the lumbar and not the entire vein. Consequently there was some admixture of general blood with the adrenal blood. This, however, would affect only the epinephrin concentrations, not the measured output. This was the only animal in which ligation of the lumbar veins was incomplete.

Specimen II was about the same strength as V and much weaker than VIII (tracings not reproduced). II was not much different from 1:6,400,000, much weaker than 1:3,800,000, much stronger than 1:10,200,000 (confirmed by several observations; tracings not reproduced), II was stronger than III (*26*, *30*, fig. 6), decidedly stronger than 1:12-800,000 (tracing not reproduced). II was stronger than IV (*30*, *32*, fig. 6), slightly stronger than V (*30*, *34*, fig. 6), decidedly weaker than 1:5,100,000 (*48*, *52*, fig. 7).

II was assayed at 1:7,000,000, corresponding to an output of 0.0026 mgm. per minute, or 0.00030 mgm. per kgm. per minute.

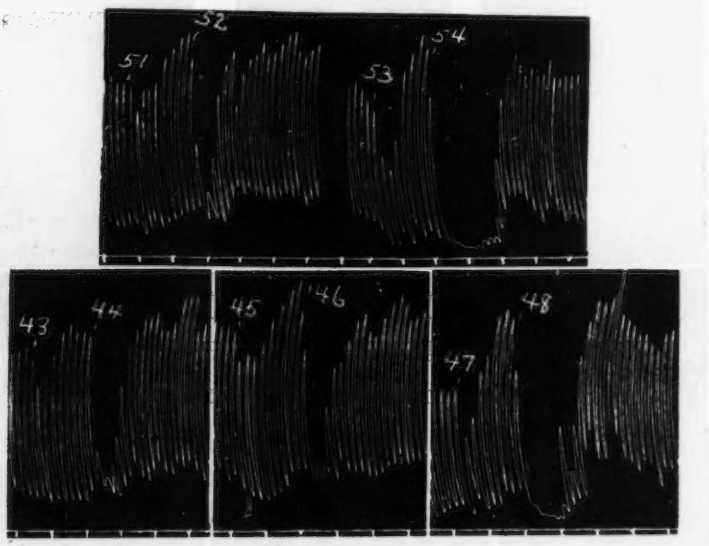

Fig. 7. Intestine tracings. Blood from dog 985. At *43*, *45*, *47*, *51* and *53* Ringer's solution was replaced by indifferent blood and this at *44*, *52* and *54* respectively by adrenal vein blood specimens V (collected during stimulation of the brachial nerves), II (collected without stimulation) and VIII (collected during splanchnic stimulation). At *46* and *48* the indifferent blood was replaced by indifferent blood to which was added adrenalin to make up concentrations of 1:6,400,000 and 1:5,100,-100 respectively. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-fifths.

III was taken at 1:9,000,000, giving an output of 0.0023 mgm. per minute, or 0.00026 mgm. per kgm. per minute.

IV, collected during brachial stimulation, was weaker than III (*26*, *32*, fig. 6) and was taken at 1:10,000,000, corresponding to 0.0019 mgm. per minute, or 0.00022 mgm. per kgm. per minute.

V, collected during off and on stimulation of the brachial, was probably slightly weaker than II (*30*, *34*, fig. 6) and slightly weaker than VI (*42*, fig. 6 and *44*, fig. 7), much weaker than 1:5,100,000, somewhat stronger than 1:6,400,000, (*44*, *46*, *48*, fig. 7). V was somewhat weaker than II in an observation (tracing not reproduced) immediately preceding *52*, fig. 7.

V was assayed at 1:7,500,000, giving 0.0027 mgm. per minute, or 0.00030 mgm. per kgm. per minute.

VI was much weaker than VII (*36*, *42*, fig. 6), or than VIII (tracings not reproduced), slightly stronger than V (*42*, fig. 6; *44*, fig. 7), much weaker than 1:5,100,000 (*42*, fig. 6; *48*, fig. 7), not far from 1:6,400,000. It was taken at 1:6,500,000, corresponding to an output of 0.0025 mgm. per minute, or 0.00028 mgm. per kgm. per minute.

Specimen VII, collected during stimulation of the splanchnic, was far stronger than any of the other specimens (*36*, fig. 6), much stronger than

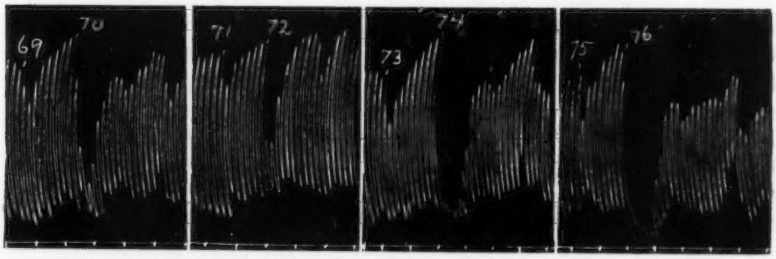

Fig. 8. Intestine tracings. Blood from dog 985. At *69*, *71*, *73* and *75* Ringer's solution was replaced by indifferent blood and this at *70* and *74* respectively by adrenal vein blood specimens VIII and VII (collected during stimulation of the splanchnic) diluted with 1 volume of indifferent blood. At *72* and *76* the indifferent blood was replaced by indifferent blood to which adrenalin was added to make up concentrations of 1:7,600,000 and 1:2,550,000 respectively. All the bloods were diluted with 3 volumes Ringer, the adrenalin bloods after adding the adrenalin, and bloods VII and VIII after the addition of 1 volume of indifferent blood. Reduced to one-half.

1:3,800,000 (tracings not reproduced). VII diluted with 1 volume of indifferent blood was somewhat weaker than 1:3,800,000 (tracings not reproduced), i.e., VII was somewhat weaker than 1:1,900,000. VII was stronger than 1:2,550,000 (tracings not reproduced). VII, diluted with 1 volume of indifferent blood, was decidedly stronger than VIII, diluted with 1 volume of indifferent blood (*70*, *74*, fig. 8), but much weaker than 1:2,550,000 (*74*, *76*, fig. 8), i.e. VII was much weaker than 1:1,275,000.

VII was assayed at 1:2,000,000, representing an output of 0.0087 mgm. per minute, or 0.0010 mgm. per kgm. per minute.

Specimen VIII, collected during off and on stimulation of the splanchnic, was much stronger than any of the other specimens except VII (*54*, fig. 7 and other tracings not reproduced). It was found to be somewhat stronger than 1:3,800,000 and somewhat weaker than 1:2,550,000 (tracings not reproduced). VIII diluted with 1 volume of indifferent blood gave about the same reaction as 1:6,400,000, but was probably somewhat stronger, i.e., VIII was probably somewhat stronger than 1:3,200,000 (tracings not reproduced). VIII diluted with 1 volume of indifferent blood was decidedly stronger than 1:7,600,000 (*70*, *72*, fig. 8), i.e., VIII was decidedly stronger than 1:3,800,000. It was assayed at 1:2,750,000, corresponding to an output of 0.0088 mgm. per minute, or 0.0010 mgm. per kgm. per minute.

In this experiment the initial output was 0.00030 mgm. per kgm. per minute (0.00026 mgm. just before stimulation of the brachial nerves). During stimulation of the brachial there was no increase whatever in the output (0.00022 mgm. to 0.00030 mgm. per kgm. per minute). No reflex stimulation of the epinephrin-secretory fibers n the splanchnic had therefore been produced, although the usual signs of effective stimulation of the afferent nerves were very evident. The result was quite different when the efferent secretory fibers were directly stimulated. During stimulation of one splanchnic the output was almost trebled. If both splanchnics had been stimulated the output would have been 6 times the initial output.

Before leaving this experiment it would seem useful once more to emphasize the point that with blood containing high concentrations of epinephrin, giving reactions approaching the maximum of which the segment is capable, it is indispensable, in order to secure anything like accuracy in the assay, to dilute the adrenal vein blood with indifferent blood.

The results in 5 of the other dogs being precisely the same, and arrived at in the same manner, it will suffice to present them in tabular form (table 1).

To illustrate the experiments on cats, two protocols will be cited and a small sample of the tracings reproduced. One of the animals was anesthetized with ether, the other with urethane.

Condensed protocol. Cat 983. Female. Weight 2.2 kgm. Ether. At 9:30 a.m. cava pocket completed and cannula inserted.

9:42 a.m. Specimen I collected, 2.51 grams in 30 seconds (5.02 grams per minute). Blood pressure 116 mm. Hg.

9:42½ a.m. Specimen II, 4.13 grams in 1 minute. Now put electrodes on the brachial, including the median nerve, and inserted a fresh cannula into cava. Discarded blood for 30 seconds. Blood pressure 108 mm.

9:57 a.m. Specimen III collected, 1.67 gram in 30 seconds (3.34 grams per minute). Stimulation of brachial begun 8 seconds before end of collection (coils 8 cm. apart). Blood pressure 86 mm.

9:57½ a.m. Specimen IV collected with stimulation of brachial (continuous), 1.44 gram in 30 seconds (2.88 grams per minute). Blood pressure 78 mm.

9:58 a.m. Specimen V collected during stimulation of brachial (off and on), 2.28 grams in 1 minute. Blood pressure 76 mm. Anesthesia was light; marked effect produced by stimulation on respiratory movements, skeletal reflexes, etc. A fresh cannula was now put into the cava and electrodes were placed on the left splanchnic. Blood was discarded for 30 seconds.

10:19 a.m. Specimen VI collected during stimulation of the splanchnic (coils 9 cm.), 1.93 grams in 1 minute. Indifferent blood then obtained from the abdominal aorta.

Left adrenal weighed 0.178 gram, right 0.180 gram.

Specimen II was somewhat stronger than I, corresponding to the somewhat smaller blood flow; both much weaker than 1:5,100,000 (tracings not reproduced). II was somewhat weaker than 1:10,800,000; I and II

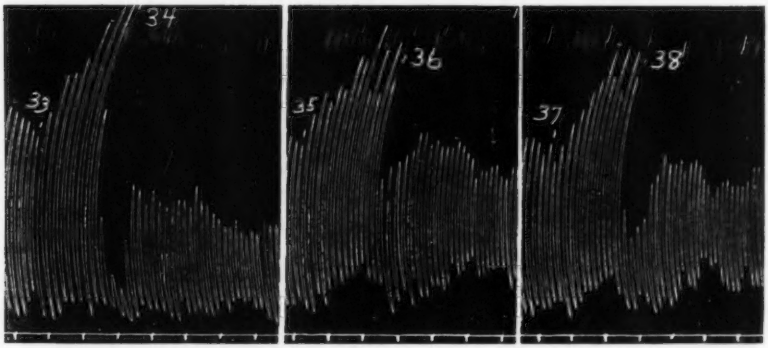

Fig. 9. Intestine tracings. Blood from cat 983. At *33*, *35* and *37* Ringer's solution was replaced by indifferent blood and this at *34* and *36* by indifferent blood to which was added adrenalin to make up concentrations of 1:2,000,000 and 1:3,200,000 respectively. At *38* the indifferent blood was replaced by adrenal vein blood specimen VI (collected during stimulation of the splanchnic) diluted with 1 volume of indifferent blood. All the bloods were diluted with 3 volumes Ringer, the adrenalin bloods after adding the adrenalin, and specimen VI after the addition of 1 volume of indifferent blood. Reduced to three-fifths.

weaker than 1:12,800,000 (tracings not reproduced). Specimen II was taken at 1:14,500,000, giving an output of 0.00028 mgm. per minute, or 0.00013 mgm. per kgm. per minute.

Specimen III was much weaker than 1:6,400,000, not very different from 1:12,800,000 (*46*, *48*, fig. 10). Another pair of tracings taken from a more sensitive segment showed that III was weaker than 1:12,800,000 (tracings not reproduced). Taking it at 1:13,000,000 we get 0.00026 mgm. per minute, or 0.00012 mgm. per kgm. per minute.

Specimen IV, collected during stimulation of the brachial, was no stronger than III (*44*, *46*, fig. 10). It gives a very weak reaction, much less than 1:6,400,000 adrenalin (*42*, fig. 10) or than specimen VI diluted with 1 volume of indifferent blood (*38*, fig. 9). Earlier in the assay, when the segment was somewhat less sensitive, IV gave no reaction,

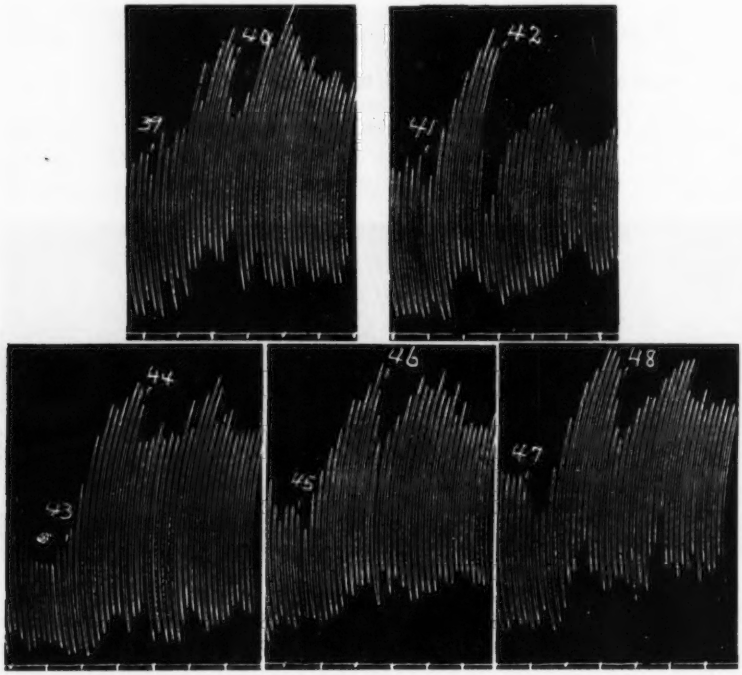

Fig. 10. Intestine tracings. Blood from cat 983. At *39*, *41*, *43*, *45* and *47*, Ringer's solution was replaced by indifferent blood and this at *40* and *44* by adrenal vein blood specimens V and IV respectively (collected during stimulation of brachial nerves). At *46* the indifferent blood was replaced by adrenal vein blood specimen III (collected without stimulation of nerves). At *42* and *48* the indifferent blood was replaced by indifferent blood to which was added adrenalin to make up concentrations of 1:6,400,000 and 1:12,800,000 respectively. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-fifths.

while that produced by VI was enormous and fully as great as that caused by 1:1,280,000 adrenalin (*22*, *24*, *26*, fig. 11). The difference in the effect upon the segment of the real epinephrin liberated through direct splanchnic stimulation and of the imaginary epinephrin liberated reflexly through stimulation of the afferent nerves must strike every reader.

IV was taken at 1:13,000,000, corresponding to an output of 0.00022 mgm. per minute, or 0.00010 mgm. per kgm. per minute.

Specimen V, collected during stimulation of the brachial nerves, was very weak compared with VI diluted with 1 volume of indifferent blood, or with 1:6,400,000 adrenalin (*38*, fig. 9; *40*, *42*, fig. 10). It was no stronger than 1:12,800,000 (*40*, *48*, fig. 10). Taking V at 1:13,000,000, we get 0.00018 mgm. per minute, or 0.00008 mgm. per kgm. per minute.

Specimen VI, obtained during stimulation of the splanchnic, was at least as strong as 1:1,280,000 (*24*, *26*, fig. 11). As the reaction with the undiluted blood was too great for a good assay it was diluted with 1 volume of indifferent blood, and then was about equal to 1:2,560,000, i.e., VI was about the same as 1:1,280,000 (tracings not reproduced).

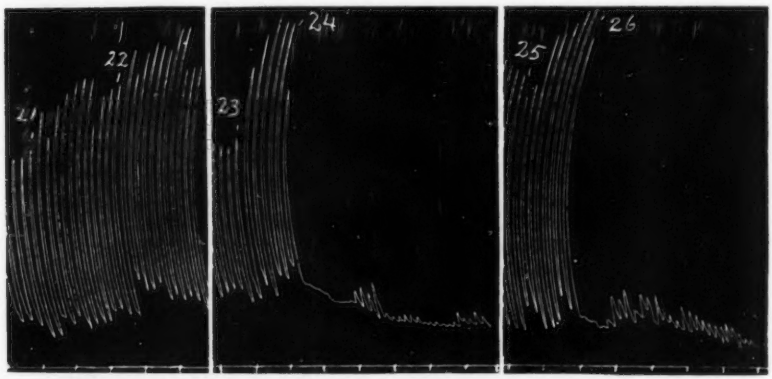

Fig. 11. Intestine tracings. Blood from cat 983. At *21*, *23* and *25* Ringer's solution was replaced by indifferent blood and this at *22* by adrenal vein blood specimen IV (collected during stimulation of brachial nerves), at *24* by specimen VI (collected during stimulation of the splanchnic) and at *26* by indifferent blood to which was added adrenalin to make a concentration of 1:1,280,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-fifths.

VI, diluted with 1 volume of indifferent blood, was much weaker than 1:2,000,000, and decidedly stronger than 1:3,200,000 (*34*, *36*, *38*, fig. 9), i.e., VI was much weaker than 1:1,000,000 and decidedly stronger than 1:1,600,000. VI was taken at 1:1,250,000, giving an output of 0.0013 mgm. per minute, or 0.0006 mgm. per kgm. per minute.

In this animal, although the initial output (0.00013 mgm. per kgm. per minute) was considerably below the average and the conditions therefore favorable for an increase, no increase whatever was caused by stimulation of afferent nerves. Direct stimulation of one splanchnic, however, increased the output nearly 5 times as compared with the initial output (more than 8 times if both splanchnics had been stimulated).

Condensed protocol. Cat 979. Male. Weight 3.05 kgm. Urethane. Cava pocket completed and cannula inserted at 10:00 a.m.

10:05 a.m. Specimen I collected, 2.40 grams in 30 seconds (4.80 grams per minute). Blood pressure 142 mm. Hg.

10:05½ a.m. Specimen II, 4.93 grams in 1 minute. Blood pressure 136 mm. Central end of left brachial nerves, including the median, now put on electrodes. Fresh cannula in cava; first blood discarded.

10:20 a.m. Specimen III, 5.02 grams in 1 minute. Blood pressure 130 mm. No stimulation of brachial till 8 seconds before end of collection of specimen III.

10:21 a.m. Specimen IV, with stimulation of brachial (coils 9 cm.), 4.00 grams in 1 minute. The blood pressure fell from 127 mm. to 96 mm., immediately following beginning of stimulation, and then recovered to 110 mm. The respiratory and other signs of effective stimulation were present.

10:22 a.m. Specimen V, with off and on stimulation of brachial (coils 10 cm.), 3.88 grams in 1 minute. Blood pressure 98 mm.

10:23 a.m. Specimen VI, without stimulation, 3.93 grams in 1 minute. Blood pressure 105 mm. Now put electrodes under left splanchnic nerve and inserted fresh cannula into cava. Discarded first blood.

10:37 a.m. Specimen VII, collected for 1 minute. No stimulation of splanchnic till 15 seconds before end of collection, 4.27 grams blood in 1 minute. Blood pressure 104 mm. till splanchnic was stimulated, when it rose sharply.

10:38 a.m. Specimen VIII, collected during splanchnic stimulation (coils 10 cm. to 9.5 cm.), 5.59 grams in 1 minute. Blood pressure rose during splanchnic stimulation to 144 mm.

10:39. a.m. Specimen IX, collected without stimulation, 4.28 grams in 1 minute. Blood pressure 106 mm. Obtained indifferent blood from the aorta. Left adrenal weighed 0.250 gram, right 0.232 gram.

Details of the assay need not be given. A few of the tracings are reproduced in figure 12.

Specimen II, collected without stimulation of nerves, was taken at 1:7,500,000, corresponding to an output of 0.00066 mgm. per minute, or 0.00022 mgm. per kgm. per minute. It is shown in figure 12 (*46*, *48*) as somewhat weaker than IV. Other tracings gave little if any difference between II and IV. V (fig. 12, *54*) also was not much different from II and IV, as shown by a number of tracings not reproduced.

Specimen III, (fig. 12, *52*), collected without stimulation of nerves, was assayed at 1:6,660,000, giving an output of 0.00075 mgm. per minute, or 0.00025 mgm. per kgm. per minute.

IV, obtained during continuous stimulation of the brachial nerves, was taken at 1:6,800,000, corresponding to an output of 0.00060 mgm. per minute, or 0.00020 mgm. per kgm. per minute.

V, collected during off and on brachial stimulation, was taken at 1:7-000,000, giving an output of 0.00055 mgm. per minute, or 0.00018 mgm. per kgm. per minute.

Specimen VI (fig. 12, *56*), collected without stimulation of nerves, was assayed at 1:6,000,000, giving an output of 0.00066 mgm. per minute, or 0.00022 mgm. per kgm. per minute.

Specimen VII (fig. 12, *58*), obtained just before stimulation of the splanchnic, was assayed at 1:6,000,000, giving an output of 0.00071 mgm. per minute or 0.00023 mgm. per kgm. per minute.

Specimen VIII (fig. 12, *60*), collected during splanchnic stimulation, was shown to be far stronger than 1:8,000,000, or than 1:5,330,000, or than any of the other specimens (tracings not reproduced), decidedly stronger than 1:4,000,000, not much different from 1:2,660,000, perhaps

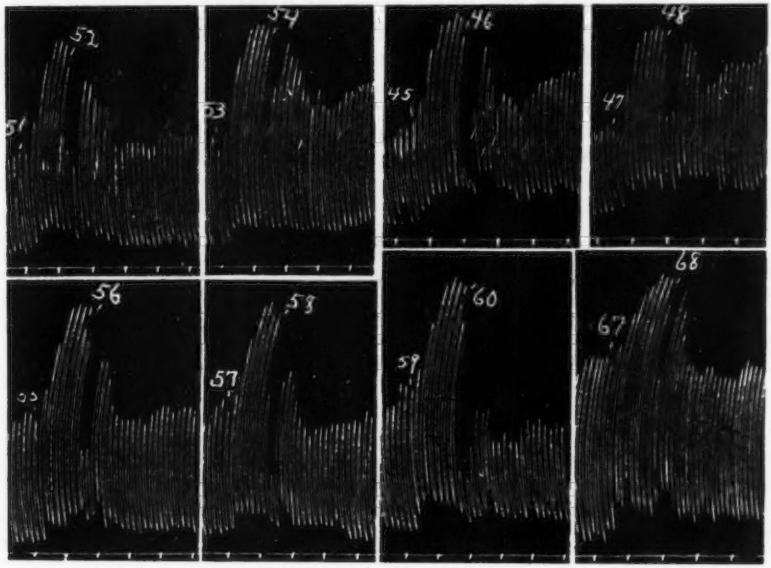

Fig. 12. Intestine tracings. Blood from cat 979. At *45*, *47*, *51*, *53*, *55*, *57*, *59* and *67* Ringer's solution was replaced by indifferent blood, and this at *46* and *54* respectively by adrenal vein blood specimens IV and V (collected during stimulation of brachial nerves), at *48*, *52*, *56*, *58* and *68* respectively by adrenal vein blood specimens II, III, VI, VII and IX (collected without stimulation of nerves), and at *60* by adrenal vein blood specimen VIII (collected during stimulation of the splanchnic nerve). All the bloods were diluted with 3 volumes Ringer. Reduced to three-fifths.

slightly weaker. It was taken at 1:2,700,000, representing an output of 0.0021 mgm. per minute, or 0.0007 mgm. per kgm. per minute.

Specimen IX (fig. 12, *68*), collected without splanchnic stimulation, was taken at 1:6,200,000, giving an output of 0.00069 mgm. per minute, or 0.00023 mgm. per kgm. per minute.

In this cat, then, no increase was caused in the epinephrin output by brachial stimulation, while direct stimulation of one splanchnic increased

it 3 times (5 times if both splanchnics had been stimulated). Considering the degree of accuracy obtainable by the method, especially with mediocre segments, no weight must be attached to an apparent small diminution in the output during brachial stimulation. Such small differences are within the limits of error and must be neglected. Ignorance of this fact, as well as of others essential to the proper employment of this method of assay, has completely vitiated the results of a recent writer (6). If an apparent diminution in the output from, say 0.00025 to 0.00020, or even 0.00018 mgm. per kgm. per minute during sensory stimulation, could not have any real significance, this is of course also true of an apparent increase of the same order, such as was seen in one animal (cat 915).

Condensed protocol. Cat 915. Male. Ether. Cava pocket completed and cannula inserted at 10:45 a.m. At 10:50 a.m. central end of the left median nerve placed on shielded electrodes.

11:00 a.m. Specimen I (without stimulation); 4.45 grams in 2 minutes (2.22 grams per minute).

11:02 a.m. II, collected during continuous stimulation of median nerve for 1 minute (coils 9 cm.); 2.19 grams in 1 minute. Respiration much increased during stimulation.

11:04 a.m. III (without stimulation), 2.93 grams in 2 minutes (1.46 grams per minute).

11:06 a.m. IV (with continuous stimulation for 1 minute); 1.66 grams in 1 minute. Now put fresh cannula into cava.

11:15 a.m. V (no stimulation), 2.49 grams in 2 minutes (1.24 grams per minute).

11:17 a.m. VI, collected during 2 minutes with continuous stimulation (8 cm.) for first minute and off and on stimulation for second minute; 1.77 grams in 2 minutes (0.89 gram per minute). Marked effect on respiration, etc., during stimulation. Now obtained indifferent blood from aorta.

A few of the tracings are given in figure 13, merely for qualitative comparison of different blood specimens. The figure illustrates again the fact more than once pointed out by us, that the mere strength of a reaction gives no information as to the output unless the rate of blood flow is known. Thus VI gave a much greater reaction than III (*14*, *22*, fig. 13), but the output corresponding to VI was not greater than that corresponding to III.

I (collected without nerve stimulation) was taken at 1:13,500,000, giving an output of 0.00017 mgm. per minute, or 0.00007 mgm. per kgm. per minute. This is much below the average output.

II, collected during median nerve stimulation, was assayed at 1:10,000-000, giving an output of 0.00022 mgm. per minute, or 0.00009 mgm. per kgm. minute.

III, collected without nerve stimulation, had a concentration of 1:4,500,000 (the blood flow was declining rather rapidly), corresponding

to an output of 0.00032 mgm. per minute, or 0.00013 mgm. per kgm. per minute.

IV, obtained during median nerve stimulation, was taken at 1:3,750,000, giving an output of 0.00044 mgm. per minute, or 0.00018 mgm. per kgm. per minute.

V, collected without stimulation (8 minutes after IV), was assayed at 1:3,700,000, giving an output of 0.00034 mgm. per minute, or 0.00014.

VI, collected during stimulation of the median nerve, had a concentration of 1:3,000,000, corresponding to an output of 0.00029 mgm. per minute, or 0.00012 mgm. per kgm. per minute.

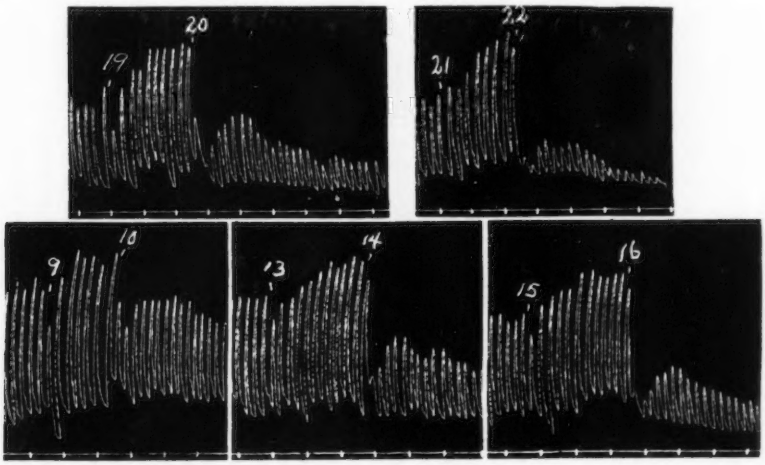

Fig. 13. Intestine tracings. Blood from cat 915. At *9*, *13*, *15*, *19* and *21* Ringer's solution was replaced by indifferent blood and this at *10*, *16* and *22* respectively by adrenal vein blood specimens II, IV and VI (collected during stimulation of the left median nerve). At *14* and *20* the indifferent blood was replaced by adrenal vein blood specimens III and V respectively (collected without stimulation of nerves). All the bloods were diluted with 3 volumes Ringer. Reduced to three-fifths.

In this animal stimulation of the afferent nerves caused no effect upon the output of epinephrin which was clearly beyond the limits of error, especially with a rather poor segment. Only a rash or an ignorant observer could see in the apparent small increase in specimen IV evidence of a reflexly induced "outburst" of epinephrin. It may be remarked here that in general it was in experiments in which the assays were particularly sharp that the lack of response of the epinephrin output to reflex stimulation was especially evident. With a good segment one can often be certain of smaller differences than the apparent differences just alluded to in cat 915, although with a mediocre segment this may not be

TABLE 1

Experiments not given in detail in text

NUMBER OF ANIMALS	SEX	BODY WEIGHT	WEIGHT OF ADRENALS	NUMBER OF SPECIMENS	BLOOD FLOW			EPINEPHRIN CONCENTRATION	EPINEPHRIN OUTPUT		REMARKS
					Grams	Seconds	Grams per minute		Per minute	Per kilogram per minute	
		kgm.	*gm.*						*mgm.*	*mgm.*	
978	F.	3.4	0.540	I	2.18	30	4.36				
				II	4.96	60	4.96	1:7,500,000	0.0066	0.00019	No stimulation
				III	2.83	30	5.66				No stimulation. III weaker than II
				IV	3.64	30	7.28	1:13,500,000	0.0054	0.00016	Stimulation of median
				V	3.07	30	6.14	1:9,300,000	0.0066	0.00019	Stimulation of median
				VI	2.16	30	4.32	1:8,000,000	0.0054	0.00016	No stimulation
				VII	1.95	30	3.90	1:10,000,000	0.00039	0.00012	No stimulation (16 minutes between VI and VII)
				VIII	3.37	30	6.74	1:3,500,000	0.0019	0.00056	Stimulation of splanchnic
				IX	2.83	30	5.66				Stimulation of splanchnic. IX somewhat weaker than VIII
				X	1.48	30	2.96				No stimulation. X stronger than VII, much weaker than IX
984	M.	6.0	0.698	I	5.21	30	10.42				No stimulation
				II	5.05	30	10.10	1:5,000,000	0.0020	0.00033	No stimulation
				III	3.84	30	7.68	1:4,000,000	0.0019	0.00032	No stimulation
				IV	4.35	30	8.70	1:4,500,000	0.0019	0.00032	Stimulation of median
				V	4.04	30	8.08	1:4,300,000	0.0019	0.00032	Stimulation of median
				VI	4.00	30	8.00	1:3,500,000	0.0023	0.00038	No stimulation
				VII	2.90	30	5.80	1:4,500,000	0.0013	0.00022	No stimulation (10 minutes between VI and VII)
				VIII	3.38	30	6.76	1:1,500,000	0.0045	0.00075	Stimulation of splanchnic
				IX	4.65	30	9.30	1:1,300,000	0.0071	0.0012	Stimulation of splanchnic
986	F.	7.3	0.858	I	5.89	30	11.78	1:12,000,000	0.0010	0.00013	No stimulation
				II	6.46	30	12.92	1:13,000,000	0.0010	0.00013	No stimulation

				III	8.46	30	16.92				Stimulation of brachial nerves. III weaker than 1:13,000,000
				IV	7.85	30	15.70	1:15,000,000	0.0010	0.00013	Stimulation of brachial nerves
				V	5.92	30	11.84	1:13,000,000	0.0009	0.00012	No stimulation (9 minutes between IV and V)
				VI	7.15	30	14.30	1:2,300,000	0.0062	0.00085	Stimulation of splanchnic
				VII	8.65	30	17.30	1:3,500,000	0.0050	0.00065	Stimulation of splanchnic
987	F.	6.5	0.766	I	5.78	30	11.56	1:12,000,000	0.00096	0.00015	No stimulation
				II	7.38	30	14.76	1:15,000,000	0.00098	0.00015	No stimulation
				III	7.74	30	15.48	1:15,000,000	0.0010	0.00015	Stimulation of median nerve
				IV	7.19	30	14.38	1:15,000,000	0.00096	0.00015	Stimulation of median nerve
				V	6.49	30	12.98	1:13,000,000	0.0010	0.00015	No stimulation (8 minutes between IV and V)
				VI	7.09	30	14.18	1:9,000,000	0.0016	0.00025	Stimulation of splanchnic (8 cm.)
				VII	8.19	30	16.38	1:6,000,000	0.0027	0.00041	Stimulation of splanchnic (6.5 cm.)
988	F.	7.3	0.852	I	5.74	30	11.48	1:12,500,000	0.00092	0.00013	No stimulation
				II	4.99	30	9.98	1:11,000,000	0.00091	0.00013	No stimulation
				III	6.70	30	13.40	1:12,500,000	0.00010	0.00014	Stimulation of branchial nerves
				IV	6.24	30	12.48	1:10,500,000	0.0012	0.00016	Stimulation of branchial nerves
				V	6.52	30	13.04	1:10,000,000	0.0013	0.00018	No stimulation (10 minutes between IV and V)
				VI	9.17	30	18.34	1:3,000,000	0.0061	0.00083	Stimulation of splanchnic
				VII	7.94	30	15.88	1:6,000,000	0.0026	0.00036	Stimulation of splanchnic
971	F.	3.16		I	2.43	60	2.43	1:10,000,000	0.00027	0.000085	No stimulation
				II	3.03	60	3.03	1:12,000 000	0.00025	0.00008	Stimulation of brachial nerves
				III	2.56	60	2.56	1:11,000,000	0.00023	0.00007	No stimulation
				IV	2.61	60	2.61				Stimulation of brachial nerves
				V	3.81	120	1.90	1:6,000,000	0.00032	0.00010	No stimulation (9 minutes between IV and V)
				VI	4.30	120	2.15	1:5,000,000	0.00039	0.00012	Stimulation of brachial
				VII	3.50	120	1.75	1:4,000,000	0.00044	0.00014	No stimulation

TABLE 1—*Concluded*

NUMBER OF ANIMALS	SEX	BODY WEIGHT	WEIGHT OF ADRENALS	NUMBER OF SPECIMENS	BLOOD FLOW			EPINEPHRIN CONCENTRATION	EPINEPHRIN OUTPUT		REMARKS
					Grams	Seconds	Grams per minute		Per minute	Per kilogram per minute	
		kgm.	*gm.*						*mgm.*	*mgm.*	
975	M.	2.39		I	1.35	30	2.70				No stimulation
				II	4.40	120	2.20	1:10,000,000	0.00022	0.00009	No stimulation
				III	2.39	60	2.39	1:8,000,000	0.00029	0.00012	Stimulation of brachial nerves (9 cm.)
				IV	2.26	60	2.26	1:7,500,000	0.00030	0.00012	Stimulation of brachial (8 to 7 cm.)
				V	3.72	120	1.86	1:7,500,000	0.00025	0.00010	No stimulation (12 minutes between IV and V)
				VI	1.87	60	1.87	1:7,000,000	0.00027	0.00011	Stimulation of brachial (7 to 6 cm.)
				VII	2.83	120	1.42	1:4,000,000	0.00036	0.00015	No stimulation
976	F.	2.82	0.402	I	1.42	30	2.84				No stimulation
				II	4.31	90	2.87	1:6,500,000	0.00044	0.00016	No stimulation (14 minutes between II and III)
				III	2.17	30	4.34	1:7,500,000	0.00058	0.00021	Stimulation of median (9 cm.)
				IV	3.80	60	3.80	1:5,000,000	0.00076	0.00027	Stimulation of median (9 cm.)
				V	4.48	60	4.48	1:6,000,000	0.00075	0.00027	No stimulation (5 minutes between IV and V)
				VI	4.44	60	4.44	1:5,500,000	0.00080	0.00028	Stimulation of median (9 cm.)
				VII	3.66	45	4.88	1:6,000,000	0.00081	0.00028	Stimulation of splanchnic
980	M.	3.15	0.494	I	2.1	30	4.2				No stimulation
				II	3.7	60	3.7	1:15,000,000	0.00025	0.00008	No stimulation
				III	3.4	60	3.4	1:14,000,000	0.00024	0.00008	No stimulation
				IV	3.75	60	3.75	1:13,500,000	0.00028	0.00009	Stimulation of median (8 cm.)
				V	3.3	60	3.3	1:12,000,000	0.00027	0.00009	Stimulation of median (6 cm.)
				VI	2.5	60	2.5	1:10,000,000	0.00025	0.00008	No stimulation (6 minutes between VI and VII)
				VII	3.0	60	3.0	1:800,000	0.0037	0.0012	Stimulation of splanchnic (8 cm.)
				VIII	2.2	60	2.2	1:1,500,000	0.0015	0.00048	No stimulation

982	M.	2.18		I	0.87	30	1.74				No stimulation. Deep urethane anesthesia
				II	2.34	90	1.56	1:10,500,000	0.00015	0.00007	No stimulation
				III	2.22	90	1.48	1:10,500,000	0.00014	0.000065	No stimulation (15 minutes between II and III)
				IV	2.47	120	1.23	1:9,000,000	0.00014	0.000065	Stimulation of brachial nerves (8 to 5 cm.)
				V	2.25	120	1.12	1:8,000,000	0.00014	0.000065	Stimulation of brachial nerves (8 to 6 cm.)
				VI	1.96	120	0.98	1:7,500,000	0.00013	0.00006	No stimulation

The first 5 animals in the table are dogs, the rest cats.

possible. With a rather insensitive or inconstant segment the greater concentrations associated with a declining blood flow can usually be better assayed than weaker concentrations corresponding to a larger blood flow.

The essential data of the remaining experiments on cats are given in table 1. It is to be understood that the experiments were made according to the same plan as those whose protocols have been given. When two periods of stimulation of nerves succeed each other, the first was continuous, and the second off and on stimulation.

In cat 976 stimulation of the splanchnic caused no change in the output, although, as the nerve was stimulated in the continuity, it was evident that the afferent fibers were being effectively excited, from the increase in respiration, etc. It is impossible to know whether the efferent epinephrin-secretory fibers had been injured, But the observation at any rate furnishes confirmatory evidence that afferent stimulation did not alter the output.

The only conclusion we can draw from the experiments described in this paper is the same as we came to in our previous work (1), (2), (3), (4), namely that it has proved impossible to demonstrate any increase in the output of epinephrin produced reflexly by stimulation of afferent nerves.

The contrary conclusion arrived at by Kodama (6) is, as already pointed out, based upon an inadequate grasp of the procedure by which we estimate the epinephrin output. The masses of alleged concentrations and outputs with which he has filled his papers have no relation to realities. Concentrations from 1:200,000 to 1:400,000 are quite common. He frequently finds 1:100,000 to 1:200,000, and even gives a concentration as high as 1:70,000 in adrenal vein blood with a good rate of flow through the adrenals. It would be a very simple matter to estimate such concentrations colorimetrically, and the intestine segment method would be quite superfluous. We should advise anyone who really thinks that Kodama's figures are not purely fanciful to try what he can get with the colorimetric method of Folin, Cannon and Denis applied to adrenal vein blood. A glance at our table 1, or corresponding results in our previous papers will show that it would be hopeless for us to make the attempt.

Naturally from these prodigious concentrations, Kodama deduces prodigious rates of output of epinephrin, so great that in some of his experiments an amount equivalent to the whole store of epinephrin in the adrenals would be formed and discharged in little more than half an hour. It would be very unprofitable to spend time in analyzing these imaginary figures. With such high concentrations it would be impossible for us to assay the blood without diluting it greatly with indifferent blood, according to our practice. Otherwise we should be trying to compare immensely strong or maximal reactions. Yet the Japanese writer does not once mention that he ever had occasion to dilute the adrenal blood in this way. It is indeed curious how relatively feeble are the intestine reactions which he professes often to obtain with what we should consider enormous concentrations, and this although he atropinized all his segments at the beginning, and atropin usually increases

the sensitiveness of the segment for epinephrin. We never use atropin when the segment is acting well without it. But Kodama appears to view and to compare with equal complacency reactions obtained on segments so insensitive that 1:250,000 adrenalin produces only a very moderate effect, and maximal reactions obtained with normally sensitive segments. He has too many specimens, and so far as we can judge from the numbers on the tracings, does not take nearly enough tracings to assay them. In short, he usually makes his comparisons in a way which we should consider unjustfiable, and neglects the points which we consider essential. Frequently he makes out a difference, even a large one, in the concentrations of two specimens when we should consider them practically identical, and not seldom he concludes that what his own curves would show to be the stronger of two specimens, is the weaker.

All his blood specimens were drawn off through one cannula, which often got clogged and was cleaned out from time to time. It is far better to insert fresh cannulae. It is absolutely not permissible to use for calculating the output of epinephrin, two consecutive blood specimens in which the flow varies enormously, obviously owing to clotting in the cannula, while the alleged concentration remains practically unchanged. Thus, in one experiment the blood flows for 7 consecutive samples are given as 3.2, 16.0, 7.7, 2.0, 1.54, 11.0, 4.8 cc. per minute. For the next specimen the flow was only 0.8 cc. per minute, the cannula being evidently plugged with clot. The concentrations given are practically the same for the 7 specimens, and large differences are calculated in the output by considering the blood flows as genuine. This is simply farcical. It is impossible that the flow can jump about in this way except through a varying obstruction to the outflow. Of course if one collects only a seventh of the blood really belonging to a particular sample and then calculates the epinephrin output on this basis it will come out 7 times too small. Yet this procedure is adopted without remark. The way in which the alleged concentrations remain anchored for a long series of samples with very different rates of blood flow is curious, to say the least of it. Nor would the reader guess that there could be any question of the trustworthiness to the last figure of the numbers by which practically every vacant space in every one of the numerous tables is filled. But the fact is that even if the assays were properly made, it would be impossible to assay all the numerous specimens with the same accuracy, or to assay some of them at all.

It would be easy to point out many instances of all the shortcomings alluded to, but it would not be profitable as too much space has already been occupied with work which is valueless because the observer has misapplied the method he relies on. We say this with sincere regret since the work has obviously consumed much time and labor.

That his results should differ widely from our own, even in such an easily verifiable matter as the average epinephrin output under the conditions of our experiments and the extreme limits observed (8), does not at all surprise us, although Kodama states that it surprised him. For we believe we were really estimating the output and he was not. It seems to us a pity that this young investigator when he was "surprised" by finding 2 or 3 times as great an average output in cats and 4 times as great an output in dogs as we found, should not have suspected that he was not using the method properly.

One other matter it is necessary to mention, and we do so with reluctance, and only to prevent a possible misapprehension on the part of a hasty reader. Since the Japanese author makes a point of having faithfully followed our method, we must state distinctly that we do not consider his experiments on stimulation of

sensory nerves in unanesthetized dogs (7) justifiable. *The results of the stimulation, on his own showing, did not differ essentially from those in the anesthetized animals.* It is scarcely an extenuation that the abdominal operative field had been partially "deafferented," although not so thoroughly as to prevent pain, for the median nerve, which he stimulated, was of course not in a "deafferented" field. All the animals in all our investigations have of course been anesthetized.

Our results on the output of epinephrin during a brief stimulation of the splanchnic are worth noting. The nerve was stimulated in the continuity, as we did not wish to interfere with the spontaneous output of epinephrin, and our object was simply to produce a real increase in output which could be compared with the effects caused by excitation of afferent nerves. A control experiment showed that the increase obtained was due solely to stimulation of the efferent fibers, and it has been shown sufficiently that in the cat and dog at any rate the splanchnic controls the epinephrin secretion only of the ipsilateral adrenal.

The rate of output in 8 dogs, with stimulation of one splanchnic was 0.0015, 0.0010, 0.00056, 0.0012, 0.00085, 0.00041, 0.00083 and 0.00063 mgm. per kgm. per minute; and in 3 cats 0.0006, 0.0007 and 0.0012 mgm. per kgm. per minute. The average for the 11 animals was 0.00082 mgm. per kgm. per minute, or on the assumption that both splanchnics had been stimulated, 0.0014 mgm. This is approximately 7 times the average output of animals anesthetized with ether (0.0001 mgm. per kgm. per minute for each adrenal), and would suffice to maintain a concentration of epinephrin in the arterial blood of the order of 1:100,000,000 to 1:150,000,000. That this is approximately correct is confirmed by considering the concentrations of epinephrin estimated in the adrenal vein blood during splanchnic stimulation, remembering that these must all be nearly doubled for the case of stimulation of both splanchnics. The concentrations may often be in the neighborhood of 1:1,000,000 or even greater, and the adrenal blood is diluted at least 100 times in the general circulation. This is apparent also from a comparison of the measured blood flows from the adrenals with the output of the heart per minute.

Calculating the initial output in milligrams per kilogram per minute and the output during splanchnic stimulation for the excited adrenal in the different experiments, we obtain the following values: dog 981, 0.00013, 0.0013 (10 times); dog 985, 0.00015, 0.00085 (6 times); dog 978, 0.00019, 0.00047 (2½ times); dog 984, 0.00016, 0.00059 (4 times); dog 986, 0.000065, 0.00079 (12 times); dog 987, 0.000075, 0.00034 (4½ times); dog 988, 0.000065, 0.00076 (12 times); dog 989, 0.00023, 0.00050 (2 times); cat 983, 0.000065, 0.00053 (8 times); cat 979, 0.00011, 0.00059 (5 times), cat 980, 0.00004, 0.00116 (29 times).

Included in these results is one experiment (on dog 989), in which a direct comparison was made between the effect on the epinephrin output

of stimulating the central and the peripheral ends of the splanchnic. The animal, a male, weighing 5.6 kgm., was anesthetized with ether. The blood pressure being 132 mm. of mercury, an adrenal vein blood specimen was obtained in the usual way (5.26 grams in 30 seconds, or 10.52 grams per minute) with both adrenals discharging. It was assayed at 1:4,000,000 adrenalin, corresponding to an output of 0.0026 mgm. per minute, or 0.00047 mgm. per kgm. per minute. The right adrenal veins, including the lumbar at the outer edge of the gland, were then tied, and another sample of adrenal vein blood collected from the left gland alone (2.73 grams in 30 seconds, or 5.46 grams per minute). It was also assayed at 1:4,000,000, giving 0.00136 mgm. per minute or 0.00024 mgm. per kgm. per minute. The right splanchnic was now stimulated in the continuity and two consecutive adrenal blood specimens collected (of course from the left adrenal only) for 30 seconds each (4.98 and 5.42 grams per minute respectively). The two specimens were assayed at 1:3,500,000 adrenalin, giving outputs of 0.00142 mgm. per minute, or 0.00025 mgm. per kgm. per minute; and 0.00155 mgm. per minute or 0.00027 mgm. per kgm. per minute respectively. Stimulation of the right splanchnic therefore caused no change in the output from the left adrenal, although the signs of effective excitation of the afferent fibers were present, including a rise of blood pressure from 112 to 138 mm. of mercury. Afferent stimulation in this nerve field has accordingly no more influence than in the other fields investigated.

The left splanchnic was now cut and its peripheral end stimulated during collection of two consecutive adrenal blood specimens (2.80 grams in 30 seconds, or 5.60 grams per minute; and 3.96 grams in one minute, respectively). The first of these two specimens was assayed at 1:2,000,000 corresponding to an output of 0.0028 mgm. per minute or 0.00050 mgm. per kgm. per minute; and the second at 1:1,600,000, giving an output of 0.0025 mgm. per minute, or 0.00045 mgm. per kgm. per minute. Stimulation of the peripheral end of the splanchnic therefore doubled the output from the corresponding adrenal.

The calculated rates of output during splanchnic stimulation may be below rather than above the true values. For there is reason to believe that the effective period of stimulation may differ from the nominal period, the increased output perhaps considerably outlasting a five-seconds' stimulation and tailing off long before the end of a much longer nominal time of excitation. That is one reason why such relatively high rates may be obtained with stimulation periods lasting only a few seconds, as seen in previous experiments of ours (10), (11) in which we attempted to determine approximately the rate of output with splanchnic stimulation by means of the denervated eye and blood pressure reactions. For these very short periods another factor may exaggerate the apparent rate of output, namely, the sudden pushing on into the circulation of the blood already in the adrenals and containing the higher concentration of epinephrin appropriate to the previous

slower blood flow. The more rapid entrance into the capillaries of the reacting tissues of the column of blood with an increased epinephrin concentration may also increase the reaction. Further, it is impossible to inject the adrenalin solution employed to imitate the reaction at the same rate as the epinephrin coming from the adrenals. Even when the adrenalin is injected for a period equal to the nominal period of stimulation the injection time may really be greater than the time of effective stimulation, and the dose of adrenalin required to imitate the reaction may be too high. No such difficulties exist when the blood is collected and assayed on intestine segments. The total quantity of epinephrin given off during the nominal period of stimulation can be determined, although it cannot be known without special experiments whether it was liberated uniformly throughout the stimulation period. It must therefore be pointed out that if the quantities of epinephrin given off during the nominal stimulation times employed by us were really liberated mainly or entirely in the first portion of those periods, the concentrations of epinephrin produced in the arterial blood for a brief time would be greater than those calculated on the assumption that the increased output was uniformly distributed over the whole time of stimulation.

Tournade and Chabrol have recently stated that the mean output of epinephrin from one adrenal is 0.05 mgm. during excitation of the splanchnic for a minute in a 7 to 9 kgm. dog. This is about 0.006 mgm. per kgm. per minute. They calculate that this would give a concentration of epinephrin in the whole of the arterial blood of at most 1:10,000,000. They merely consider the epinephrin as distributed over the total quantity of blood in the body, ignoring the fact that in dogs of this size the blood will complete the round of the circulation more than once in the minute. The monstrous concentration of 1:10,000,000 (1:5,000,000, if both adrenals were to be excited simultaneously) could of course be easily detected and estimated in the arterial blood by the segment method. They quote Battelli's old estimate (12), based on an untrustworthy method, of 1:10,000,000 to 1:20,000,000 in the arterial blood of dogs under ordinary conditions without stimulation of the splanchnics. They consider that this estimate is too high, but do not seem to realize that it is probably at any rate 50 to 100 times too high. The estimate of Tournade and Chabrol of the epinephrin given off from the adrenal with splanchnic stimulation seems excessive, at least 7 times too high if the increase is considered as uniform over the whole period of stimulation. If, however, the whole of the increased output, or the greater part of it, occurs in the first fraction of the period, the rate of output for that time may be considerably above the average rate over the whole period. As already pointed out, injection of adrenalin over a period equal to that of the stimulation does not necessarily imitate closely the actual discharge from the gland if this takes place mainly or solely during the first part of the nominal stimulation period.

If Tournade's estimates were correct for periods as long as a minute, we ought to find concentrations of 1:100,000 or more in the adrenal vein blood, which are very different from the concentrations which we do find. The concentration of 1:10,000,000 calculated by the French authors for the arterial blood is at any rate 15 to 20 times too great. The ingenious experiments which they have multiplied, in order to prove that when the splanchnic is stimulated epinephrin is given off in sufficient amount to cause physiological reactions, seem sometimes to be superfluous, since for a considerable time it has been known that a whole series of reactions can be produced in this way (reactions of the denervated eye, blood pressure reactions, heart reactions, to which Tournade has added others). It is true that Gley seems to doubt this, but on insufficient grounds. Tournade and Chabrol

are perfectly correct in pointing out that the failure of arterial blood to elicit given reactions is no proof of the absence of epinephrin in it. Once the magnitude of the output caused by splanchnic stimulation is established it ought to be possible to decide whether this amount of epinephrin can or can not elicit a given reaction.

SUMMARY

We have confirmed by new experiments on 8 dogs and 8 cats our previous result on the effect of stimulation of afferent nerves on the rate of output of epinephrin from the adrenals. In none of the experiments was any increase demonstrated by the rabbit intestine segment method.

When, instead of attempting to cause reflex stimulation of the epinephrin-secretory fibers, we stimulated them directly in the splanchnic nerve, the result was quite different. The average output per kilo of body weight per minute with stimulation of one splanchnic in 11 animals was 0.0008 mgm., 4 times the average output in animals anesthetized with ether without splanchnic stimulation. The average increase for the stimulated adrenal was therefore from 0.0001 to 0.0007 mgm. per kgm. per minute. With stimulation of both splanchnics the increase would have been from 0.0002 to 0.0014, sufficient to increase the epinephrin concentration in the arterial blood from, say, 1:1,000,000,000 to 1:140,000,000, in individual cases still more. The initial output for one adrenal was increased in the different experiments by stimulation of the corresponding splanchnic 2 to 12 times. In one experiment, with a very low initial output, the output for the excited adrenal was 29 times its initial value. In expressing the value of the output during splanchnic stimulation as so much per minute, the assumption is made for convenience that the rate of output calculated for the actual period of stimulation (usually 30 seconds) would continue to be the same for a minute. If, however, the time of maximum efficiency of a stimulus is less than this, the rate of output for the period of maximum efficiency may be greater than the values given.

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BIOLOGICAL FOOD TESTS

VIII. Vitamins A and B in Radish

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In the course of an investigation into the vitamin A and vitamin B content of certain brightly colored vegetable foods, a few tests were made upon dried radishes. These results are presented here for whatever interest they may bear as indicating possible reasons for or against the use of one of the less important but commonly used vegetables.

The bright red color of radishes (*Raphanus Sativus* L.) like that of beets is apparently due to pigments of an anthocyanin rather than carotinoid nature. Schudel (1) isolated two pigments which he called *raphanin* and *rubin* from radish peelings by means of chloro-picric acid. These were shown on hydrolysis to be respectively a flavone diglucoside and a glucoside. These pigments are distinguished from the carotinoids by their solubility in water.

Since considerable evidence has been accumulated linking the occurrence of certain fat-soluble pigments, notably carotin, with that of vitamin A there is some interest in the inquiry as to the possible parallelism between the appearance of the water-soluble pigments and the same or another vitamin. Radishes and beets are prominent examples of highly colored vegetables which owe their color to other than carotinoid pigments. Other root vegetables known to contain carotin and xanthophylls, are carrots and sweet potatoes. These latter vegetables have been shown by Steenbock and Gross (2) to contain considerable amounts of vitamin A. Beets on the other hand have been shown in the same study to be "very low or practically free from fat-soluble vitamin." The nature of the yellow pigments of parsnips and rutabagas has not been studied. Since these roots were found to be deficient in vitamin A, the presence or absence of carotinoids in them will prove of interest as contributing to or detracting from the theory of simultaneous occurrence of these particular pigments along with vitamin A in vegetable tissues. Investigation of the nature of these pigments is under way in this laboratory. The negative results of the investigation into the vitamin A value of radishes here reported confirms our tentative theory that water-soluble

pigments, the anthocyanins, are not produced in the same vegetable tissues which contain the fat-soluble vitamin A.

No earlier vitamin study of radishes by means of rat feeding has been made so far as is known to the writer. Eddy and Stevenson (3) using the yeast growth method which they afterwards repudiated as a reliable measure of vitamin B (4) placed radishes low in the list of vegetables and fruits examined, but parallel with onions and carrots. Petragnani (5) using pigeons, placed the vegetables tested in the following decreasing order of protective value: peas, cabbage, lettuce, radishes, pears, plums. No study upon the vitamin A value of radishes has been reported.

Experimental methods. The radishes used were young, tender specimens of the long, slender variety, were ground whole and dried quickly at 60°C. The basal diet was the same as that previously described (6) and was supplemented by 1 gram of butter fat daily per rat when the vitamin B tests were being made, and with 0.3 gram of dry brewery yeast instead when the vitamin A tests were being made. This diet with both supplements has been shown many times in this laboratory to promote normal growth in rats and even to support reproduction and rearing of the young.

Absence of vitamin A in radishes. Four young rats taken at weaning were fed vitamin A-free basal diet until their rapidly falling weight and severe eye disease indicated vitamin A deficiency. This as is usual in our experiments occurred at the end of four weeks upon the experimental diet. One gram daily additions of dried radish were then given each animal. This amount, equivalent to 8 grams of the fresh vegetable, was as much as the rats could be induced to consume. No delay of decline resulted, and all the animals died within two to six weeks after the administration of radish was begun. The growth curves for these rats are shown in figure 1. Since the average intake of basal diet by these animals was 7 grams per day during the earlier part of the experiment, and not less than 4 grams daily even during the last week of life the proportion of total food intake represented by the dried radish was in all cases more than 20 per cent. It seems fair to conclude, therefore, that radishes are either very low in, or practically free from vitamin A.

Vitamin B in radishes. Four young rats taken at weaning were fed vitamin B-free basal diet until cessation of growth, unkempt fur, partial leg paralysis, and similar evidences of disturbed nutrition indicated vitamin B deficiency. This occurred after four weeks. The animals were then given one gram daily doses of dried radish. Growth was resumed, the appearance of the rats became normal, and although the sizes attained were not as great as those of control animals of the same litter fed 0.3 gram dry brewery yeast as source of vitamin B, a considerable potency as to this vitamin may in consequence be ascribed to the radish. Growth curves for these rats and for controls are shown in figure 1.

In terms of dry weight the amount of radish equivalent in vitamin B to 0.3 gram of dry yeast is 0.87 gram or, in the scale proposed by Morgan and Francis (7), 34 per cent of the standard, ++++. In terms of fresh weight approximately 8 grams of radish are required or 3.7 per cent (++++) of the standard. These figures are close to those obtained in similar manner for dehydrated pumpkin, and not much lower than those for fresh cow's milk and for orange juice.

The deficiency of radishes in vitamin A here demonstrated is not unexpected in view of the type of pigment, namely, an anthocyanin, found in this vegetable. The results obtained show complete parallelism with those of Steenbock and Gross on red beets.

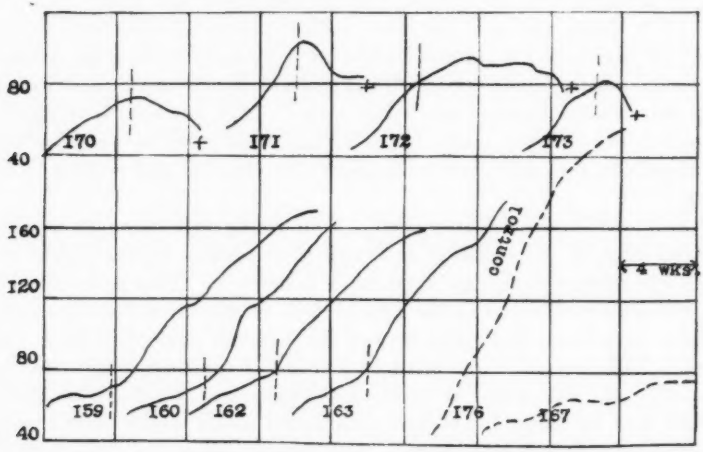

Fig. 1. Growth curves of rats fed dried radish as sole source of vitamin A or vitamin B.

Rats 170, 171, 172, 173 were given daily 1 gram of dried radish beginning at dotted line, after decline in weight and xerophthalmia had been established on vitamin A-free basal diet.

Rats 159, 160, 162, 163 were given daily 1 gram of dried radish beginning at dotted line, after decline in weight and characteristic symptoms had developed on vitamin B-free basal diet.

Rat 176 was a control of the same litter as several of the other rats shown in this chart, given 0.3 gram dry brewery yeast and 1 gram butterfat daily in addition to basal diet.

Rat 167 was an animal of the same litter as several of the others, maintained for several weeks on vitamin B-free basal diet.

SUMMARY

1. Dried radishes fed in the amount of 1 gram daily to young rats suffering from vitamin A deficiency proved inadequate to prevent decline

and death. This dosage corresponds to approximately 8 grams of fresh radishes and constituted about 25 per cent of the total food eaten. Radishes must be very low in or free from vitamin A.

2. Attention is drawn to the possible relation between the anthocyanin nature of the pigment of radish peelings and this absence of vitamin A. A similar illustration occurs in red beets.

3. Dried radishes fed in the amount of 1 gram daily to young rats suffering from decline in weight and other symptoms of vitamin B deficiency sufficed to promote nearly normal weight increases over the period of most active growth. This is equivalent to about 8 grams of fresh radish, and is not far from the amount in fresh milk or orange juice required for the same vitamin B effectiveness.

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THE RELATION BETWEEN INCREASED MUSCLE TENSION AND CREATINE

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In a study of the distribution of nitrogen in the blood of patients suffering from mental disorders recently published from this laboratory (1), it was shown that the creatine content was decreased in the groups of involution melancholia and depressed dementia praecox cases. Both these groups were made up of patients in which there is general relaxation of muscular tone and a marked diminution in interest and attention. The patients are listless and maintain themselves in a limp and flaccid condition.

The assumption was made that the creatine content of the blood was a function of the muscular tone, and that a relaxation of muscle tone if continued over a long period would result in a decrease in the creatine content of the blood. It was suggested that a corresponding increase in creatine should be found in those cases in which a marked increase in muscle tension occurred. This paper deals with the results obtained from the study of such a group of cases. Hammett (2) reports an increase in the creatine content of the blood of two cases of catatonic dementia praecox. In these patients the increase occurred while the individuals were recovering from a catatonic stupor in which there had been a marked diminution from the normal muscle tone.

Sulger (3) concludes that the amount of creatine in the muscles of cats and rabbits is some indication of the tonus. The cutting of the posterior roots causes a distinct diminution in the amount of creatine in the fixed muscles.

Uyeno and Mitsuda (4) report an increase in the creatine content of the clasping muscles of male frogs while maintained in tonic contractions during coupling. There is likewise an increased amount of creatine in the muscles while in a state of rigidity because of decerebration. They also state that the tonic contraction of muscles brought on by the injection of nicotine results in an increase in creatine.

The following is a brief report of the clinical findings in the ten cases which were studied.

Case 1, M. K., female, age forty-five, weight 102 pounds, admitted ten years ago while in a very severe catatonic stupor. At that time body and hands were flexed and could not be extended. Extremities were cold and cyanotic. Muscular rigidity has been constant since admission but not so extreme. She will respond to questions by turning head but does not speak.

Case 2, H. H., female, age forty-four, weight 100 pounds, has been mute, resistive and catatonic for four years. Is hallucinated in all spheres and has 3 plus Wassermann. The extremities are maintained in a flexed position.

Case 3, M. W., female, age fifty-nine, weight 104 pounds. Has been mute, untidy, stiff and constrained for two years during which time tube feeding has been necessary.

Case 4, S. B., female, age forty-four, weight 122 pounds, was brought to hospital in catatonic stupor seventeen years ago. She has been tube-fed, mute, untidy and in a cataleptic state the greater part of the time and has had short intervals of mild activity when there is less constraint, but at such times she is profane and abusive in reaction to hallucinations. She soon lapses again and flexibilitas cerea is very pronounced and her extremities become cold and cyanotic.

Case 5, R. M., female, age eighteen, weight 97 pounds. Three years ago she became hallucinated in the auditory and visual spheres, developed numerous morbid fears, was forgetful and absentminded. She has been mute, careless and untidy and remains in fixed attitudes. She has also shown marked flexibilitas cerea and has had to be dressed, undressed and spoon fed.

Case 6, M. C. K., female, age twenty-eight, weight 106 pounds. Six years ago she became depressed and eccentric in her conduct, was hallucinated and untidy. She assumed fixed attitudes, stared into space and seldom spoke. During this period she lost twenty pounds in weight. A year and a half ago, at the time of her admission, she became so blocked as to be unable to eat or take care of herself in anyway. During her residence she has been tube-fed twice daily. She has been mute, untidy and to some extent resistive. Muscle rigidity not very marked.

Case 7, E. A., female, age twenty-eight, weight 93 pounds. Since her admission to the hospital three years ago, she has been tube-fed twice daily almost continuously. Occasionally she would go for a few weeks during which time she ate, but has shown constraint, resistance and considerable stupor at all times. For the past six months she has been quite rigid, mute, resistive and tube-fed. She is unable to look after personal needs at all.

Case 8, E. Y., female, age twenty-nine, weight 102 pounds. Twelve years ago she had an attack of catatonic stupor lasting about three years. She improved and remained comfortable until four years ago when she experienced another attack which lasted two years. One month before her admission to the hospital, three months ago, she became excited, then stuporous and catatonic. She has been tube-fed twice daily during her residence at the hospital. She is mute, untidy, blocked and passively resistive.

Case 9, E. G., male, age thirty-four, weight 96 pounds. For the past two years patient has been mute and untidy and has had to be tube-fed. He has held himself in a very rigid condition with extremities flexed and head raised off the bed. Two weeks ago he became less rigid and at the time the specimen was taken there was no stiffness noticeable.

Case 10, C. W., male, aged twenty-three, weight 97 pounds. For one year patient has been in a state of extreme catatonic stupor. The extremities are cold and blanched. The fingers are maintained very stiff and slightly flexed. The legs and arms are held in a flexed position from which it is impossible to straighten them.

The head is held about four inches off the pillow. This is the most extreme case of ridigity in the entire group.

The specimens were taken in the morning before breakfast, the patients having received no food since the previous evening. The blood was prevented from clotting by the use of the cloth impregnated with lithium oxalate as recommended by Folin (5). The methods employed were those of Folin and Wu, and the utmose care was taken to insure the greatest possible accuracy of the results. A complete analysis was made and the undetermined nitrogen estimated in order to complete the study which is reported in the previous paper (1).

It was found necessary to modify somewhat the method for the estimation of creatine because of the lack of a suitable autoclave. This modification has been carefully checked and found to give accurate results with known amounts of creatine.

Five cubic centimeters of blood filtrate plus 1 cc. of normal HCl were placed in a test tube graduated at 25 cc. and evaporated to dryness in a water bath. Then 1 cc. more of normal HCl was added and the evaporation repeated. The residue was next dissolved in 5 cc. of water and the determination of creatine carried out as in Folin's method except that no acid was added to the standard. The picric acid must be extremely pure and Benedict's (6) method of repeated recrystallizations from benzene is recommended.

This process of evaporation takes considerable time and it is advised that it be started before any of the other determinations are made.

The controls which are given in table 1 are speciments taken from the nurses and attendants of this institution. The results are given in milligrams per 100 cc. of whole blood and in per cent of total nitrogen. Table 2 contains the results from the ten cases showing muscular tension.

On comparison of these two tables the following differences are noted. There is a marked decrease in the uric acid nitrogen from 3.67 per cent for the controls to 2.74 per cent for the catatonic cases. This decrease is in agreement with that given by Uyematsu and Soda (7), who report a change from 2.3 per cent for their controls to 1.6 for their catatonic group.

The amount of blood sugar is also somewhat lower in the catatonic group, the figure being 81.0 mgm. as compared with 89.8 mgm. for the controls.

The figures for creatine are very striking, the change being quite marked. There is an increase in the absolute amount from 6.98 mgm. per 100 cc. of blood in the controls to 11.2 mgm. for the catatonic group. The increase in terms of per cent of non-protein nitrogen corresponds to this, the figures being 8.42 per cent for the controls and 12.9 per cent for the patients.

TABLE 1

*Controls**

NUMBER	TOTAL NON-PROTEIN NITROGEN	UREA N		AMINO ACID N		URIC ACID	URIC ACID N		CREATININE	CREATININE N		CREATINE	CREATINE N		UNDETERMINED N		BLOOD SUGAR
	mgm.	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
101	34.0	17.8	52.4	6.84	20.1	2.68	0.89	2.62	1.49	0.478	1.41	6.09	2.26	6.65	5.73	16.80	93.0
102	29.0	12.0	41.4	7.66	26.3	2.65	0.88	3.04	1.33	0.427	1.47	6.06	2.25	7.78	5.77	20.00	86.0
103	34.0	18.0	52.9	8.00	23.5	3.87	1.29	3.79	1.84	0.590	1.73	7.50	2.79	8.21	3.33	9.80	84.0
104	30.8	14.9	48.4	7.11	23.1	4.21	1.40	4.54	1.53	0.491	1.60	6.60	2.46	7.98	4.44	14.49	90.0
105	29.7	15.6	52.5	6.83	23.0	3.43	1.14	3.84	1.50	0.481	1.62	6.29	2.34	7.87	3.31	11.20	89.0
106	34.5	14.7	42.7	6.93	20.1	4.40	1.47	4.27	1.70	0.545	1.58	9.22	3.43	9.93	7.43	21.40	103.0
107	31.0	20.4	65.8	5.80	18.7	3.98	1.33	4.28	1.57	0.503	1.62	7.13	2.65	8.54	0.32	1.05	82.0
108	32.4	11.7	36.1	7.49	23.1	2.87	0.96	2.96	1.48	0.475	1.47	5.97	2.22	6.85	9.55	29.50	87.0
109	30.3	10.6	35.0	6.70	22.1	2.88	0.96	3.17	1.38	0.443	1.47	5.26	1.96	6.48	9.64	31.80	92.0
110	27.7	11.7	42.3	6.25	22.6	2.88	0.96	3.46	1.49	0.478	1.76	5.62	2.09	7.55	6.22	22.30	90.0
111	25.1	9.1	36.3	6.28	25.0	2.93	0.98	3.90	1.46	0.468	1.87	5.48	2.04	8.12	6.23	24.80	99.0
112	27.4	14.7	53.7	6.97	25.4	3.29	1.10	4.02	1.34	0.430	1.57	5.94	2.21	8.07	1.99	7.26	91.0
113	34.0	12.4	36.5	6.10	17.9	3.25	1.08	3.18	1.52	0.488	1.44	8.05	3.00	8.83	10.90	32.10	92.0
114	29.4	12.4	42.2	6.31	21.5	3.21	1.07	3.64	1.77	0.578	1.97	7.15	2.66	9.04	6.38	21.65	95.0
115	29.5	11.4	38.6	6.70	22.7	2.88	0.96	3.25	1.51	0.485	1.64	8.82	3.38	11.40	6.58	22.41	88.0
116	34.0	14.6	42.9	6.34	18.6	3.27	1.09	3.21	1.53	0.491	1.44	7.55	2.81	8.27	8.67	25.58	90.0
117	36.3	21.0	57.8	6.23	17.1	4.80	1.60	4.41	1.48	0.475	1.31	7.70	2.86	7.88	4.14	11.50	86.0
118	29.0	13.4	46.3	6.41	22.1	3.50	1.17	4.04	1.42	0.456	1.57	8.22	3.06	10.60	4.50	15.40	82.0
119	29.4	13.7	46.6	7.29	24.8	3.59	1.20	4.08	1.56	0.500	1.70	7.90	2.94	10.00	3.77	12.90	87.0
Average..	30.9	14.2	45.8	6.75	22.0	3.40	1.13	3.67	1.52	0.488	1.59	6.98	2.60	8.42	5.73	18.52	89.8

* Taken from paper by author (1).

TABLE 2

Catatonic dementia praecox

NUMBER	TOTAL NON-PROTEIN NITROGEN	UREA N		AMINO ACID N		URIC ACID	URIC ACID N		CREATININE	CREATININE N		CREATINE	CREATINE N		UNDETERMINED N		BLOOD SUGAR
	mgm.	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm*
1	28.0	13.3	47.5	7.00	25.0	3.19	1.06	3.79	1.45	0.465	1.66	9.0	3.36	12.0	2.82	10.05	81.0
2	29.0	10.3	35.5	8.59	29.6	2.79	0.93	3.21	1.42	0.456	1.57	12.0	4.47	15.4	4.25	14.72	82.0
3	38.0	17.0	44.8	7.77	20.4	2.20	0.73	1.92	1.38	0.443	1.17	12.6	4.69	12.3	7.37	19.41	74.0
4	33.4	12.4	37.1	7.52	22.5	2.89	0.96	2.88	1.43	0.459	1.38	11.3	4.21	12.6	7.85	23.54	86.0
5	33.7	17.3	51.3	6.22	18.5	3.23	1.08	3.20	1.41	0.453	1.34	10.4	3.87	11.5	4.78	14.16	70.0
6	33.0	20.5	62.2	6.31	19.1	2.51	0.84	2.54	1.30	0.418	1.27	9.7	3.61	10.9	1.32	4.00	81.0
7	31.6	19.1	60.5	6.01	19.0	2.20	0 73	2.31	1.42	0.456	1.44	11.4	4.24	13.4	1.06	3.36	86.0
8	32.0	14.9	46.6	6.93	21.7	2.56	0.85	2.56	1.53	0.491	1.53	11.0	4.09	12.8	4.74	14.81	75.0
9	38.1	22.4	58.8	7.52	19.7	2.59	0.86	2.26	1.68	0.540	1.42	12.0	4.47	11.7	2.31	6.12	90.0
10	29.7	13.9	46.8	6 83	23.0	2.45	0.82	2.76	1.72	0.552	1.86	13.0	4.84	16.3	2.76	9.28	85.0
Average..	32.7	16.1	49.1	7.07	21.8	2.66	0.89	2.74	1.47	0.473	1.46	11.2	4.18	12.9	3.93	11.95	81.0

It should be noted that the highest figure both in absolute amount of creatine and in per cent of non protein nitrogen was obtained in case 10, in which the muscular tension was most marked. The lowest percentage figure in the group was obtained in case 6 in which the rigidity was not very great. In a group of eight cases of catatonic praecox not showing any muscular tension reported in the previous paper (1) the figures for creatine are 6.34 mgm. and 7.73 per cent.

The figures for these three groups are collected for comparison in table 3.

TABLE 3

	TOTAL NON-PROTEIN NITROGEN	UREA N		AMINO ACID N		URIC ACID N	URIC ACID N		CREATINE	CREATININE N		CREATINE	CREATINE N		UNDETERMINED N		BLOOD SUGAR
	mg.	*mg.*	%	*mg.*	%	*mg.*	*mg.*	%	*mg.*	*mg.*	%	*mg.*	*mg.*	%	*mg.*	%	*mg.*
Average of rigid catatonic cases.....	32.7	16.1	49.1	7.07	21.8	2.66	0.89	2.74	1.47	0.475	1.46	11.2	4.18	12.9	3.93	11.95	81.0
Average of relaxed catatonic cases...	30.7	15.3	49.7	6.96	22.1	3.42	1.14	3.77	1.61	0 518	1.70	6.34	2.36	7.73	4.54	15.2	85 4
Average of controls.....	30.9	14.2	45.8	6.75	22.0	3.40	1.13	3.67	1.52	0 488	1.59	6.98	2.60	8.42	5.73	18.5	89 8

SUMMARY

The assumption that the amount of creatine in the blood is an index of the tonicity of the muscles of the individual is supported by the findings in this paper. It has previously been shown that the amount of creatine is diminished when there is a long-continued condition of relaxation of the muscles and the figures given here show that there is a corresponding increase of creatine when there is an increase in the muscle tension over a considerable period.

I should like to express my appreciation to Drs. H. D. Purdum and J. L. Wethered of Springfield State Hospital, Sykesville, Maryland, through whose courtesy these cases were obtained.

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THE PLACE OF FATIGUE IN STRIATED MUSCLE: EVIDENCE FOR FAILURE OF EXCITATION APART FROM CONTRACTION[1]

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It has been generally held that the myoneural junction is an important seat of fatigue. Yet it has been difficult to devise a conclusive demonstration of its alleged susceptibility. The impression that it develops a resistance to the transmission of excitation when repeatedly traversed by the propagated disturbance was created long ago by the observation that a muscle can still be stimulated directly when it no longer responds to stimuli applied to its nerve. In the light of all that we have since learned about the nature of the nerve-impulse the traditional evidence is found to be untrustworthy. The impulse has a fixed magnitude (at least for a given frequency of stimulation) and when a muscle ceases to answer to indirect stimulation all that we can maintain is that a rise of threshold has taken place. The impulses which originally were adequate to initiate contraction are no longer able to do so. We cannot assert that the elevated threshold has been established at the myoneural junction, it may quite as well be a characteristic of the muscle protoplasm beyond.

The gastrocnemius of the frog has an innervation which invites experiments designed to promote the analysis of fatigue. The sciatic nerve is derived chiefly from two spinal segments. The nerves arising from these segments—IX and X according to Ecker and Wiedersheim (1)—are readily separated, even in small frogs, for a distance of 2 or 3 cm. Stimulation of either component will call the gastrocnemius into action. We have, therefore, the opportunity of comparing the responses of the muscle to separate and simultaneous stimulation of the two bundles. Furthermore, we can develop fatigue by playing upon one branch and when contraction has practically ceased we can shift to the other.

In carrying out such an experiment a nerve-muscle preparation is made and suspended in a moist chamber of the type described by Lucas (2). Each division of the sciatic is laid upon a pair of platinum electrodes. The inductorium used for stimulation has in its primary circuit a motor-driven

[1] A preliminary account of this study has appeared in Science, 1924, lix, 383.

interrupter with a device to exclude make-shocks. A thread attached to the tendon is led out of the moist chamber and moves a lever, sometimes of the isotonic and sometimes of the isometric type. When isometric contractions have been recorded the movement of the lever has been downward.

The two nerve-components are in some cases markedly unequal in size. More often they are of about the same diameter. When this is true the contractions obtained by maximal stimulation of each in turn are usually of a comparable order. If, using one nerve, we make a fatigue record

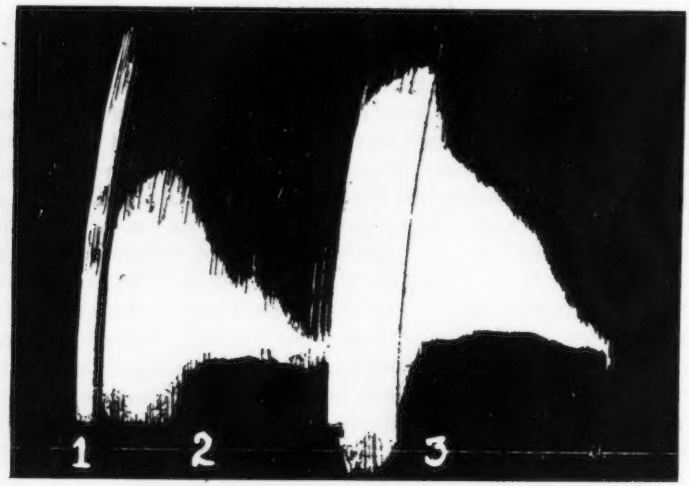

Fig. 1. The contractions recorded are isotonic. The two nerve components are stimulated as follows: *1* is a short series produced by A, *2* is a series obtained through B, and *3* represents a return to A. It will be noted that the capacity to respond to A is not reduced by the previous activity.

consisting either of successive simple contractions or a prolonged tetanus, we can be assured of obtaining a strikingly similar performance when we bring our stimulation to bear upon the second branch. It is not an overstatement to say that the second record would hardly be better if a fresh muscle were substituted. In figure 1 the contractions are isotonic. A short series, *1*, is the result of exciting one component, in this case the more effective of the two. Next a long fatigue series, *2*, is made using the second component. Finally the series *3* is obtained as a result of resuming stimulation of the first. It will be noted that a Treppe is exhibited both in *2* and *3*.

Figure 2 is a record of isometric tetanic contractions made in response to alternate stimulation of the two strands, *A* and *B*. First we have made a brief trial with *A*, then we carry fatigue to apparent exhaustion with *B*. Returning to *A* we find it capable of eliciting as much from the muscle as at first. The independence of the two is clear. Two inferences seem to be warranted by these results: *a*, that we have not commonly had a spread of current to the meeting of the two bundles, and *b*, that if fatigue products are concerned their diffusion from fiber to fiber has not been significant.

Such a capacity on the part of one muscle to develop seemingly independent contraction series when different motor-paths are employed is just what we should expect on the basis of the usual conception of the organization involved. Each nerve-fiber would be assumed to call into action its own dependent cluster of muscle-fibers. About half the contractile machinery would be thought of as resting while the other half was at work. But, as a matter of fact, we are not permitted to accept this simple explanation. We have to reconcile the observations already noted with others equally clean-cut and pointing to a very different set of conditions. We must consider the effect of stimulating both components of the nerve at the same time.

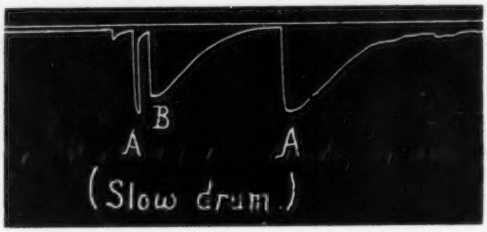

Fig. 2. The contractions are isometric and tetanic, recording downward. As in figure 1, one component is stimulated briefly at *A*, there is then a shift to *B*, and finally a return to *A* which is found as competent as at first.

The contractions of the muscle when all its motor nerve-fibers are stimulated are but little greater than when one of the strands is used alone. If this were found to be true only of isotonic contractions we might possibly harmonize it with the traditional view. It is barely conceivable, though not probable, that half the fibers of a muscle might raise a light weight as far as all the fibers could do. But the principle holds for an isometric tetanus, the intensity of which is presumably in strict proportion to the number of fibers participating. We are convinced that when we stimulate something like half the motor nerve-fibers we bring a very high proportion of the muscle-fibers into action. In figure 3 we have a comparison between the isotonic series produced by stimulating the entire nerve on one side and two series obtained by employing the two components successively on the other. In figure 4 the contractions are isometric re-

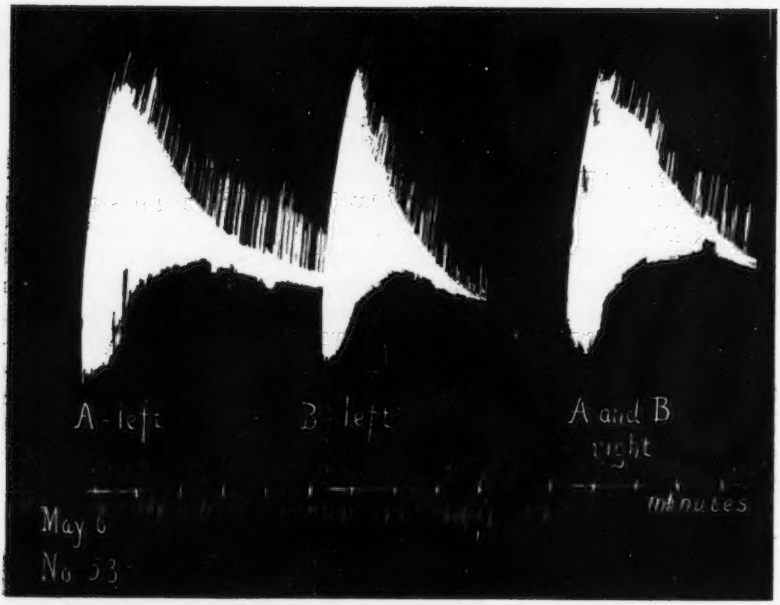

Fig. 3. Two successive series of isotonic simple contractions are made using the separate components, *A* and *B*, on one side. For comparison the entire nerve on the other side is stimulated in the same manner. The series made with all the nerve-fibers in action is but little better than either *A* or *B*.

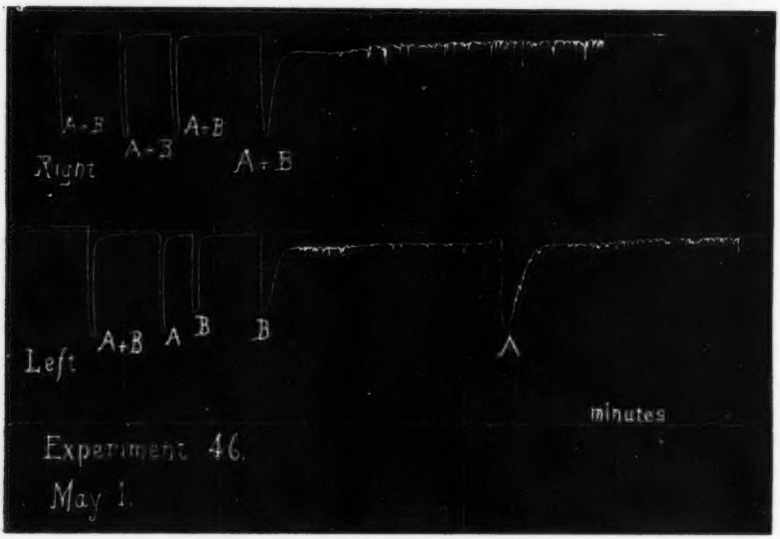

Fig. 4. The contractions are isometric and tetanic as in figure 2. The experiment is a check on that recorded in figure 3. More work is obtained by stimulating components *A* and *B* successively (lower line) than by their simultaneous excitation (above).

sponses to tetanic stimulation and again little appears to be gained by stimulating all the fibers. One may say this either of the intensity of the action or of its duration.

We have calibrated our isometric lever and typical results secured with it are assembled in table 1. The general trend of the figures supports the belief that, in round numbers, 50 per cent of the nerve-fibers may be expected to command 85 per cent of the contractile units. Figure 5 shows how little the isometric tetanus developed by stimulating both the components at once, $A + B$, exceeds that obtainable from either by itself, B, A.

The data indicate for the frog a plurisegmental innervation such as Agduhr (3) has shown to exist in cats and rabbits. As depicted by him a typical muscle-fiber has upon it two myoneural junctions and the nerve-fibers thus connected with it have come from different segments of the

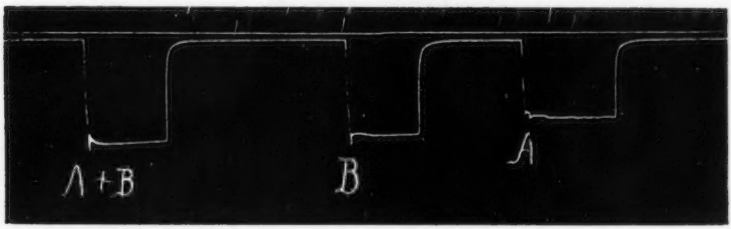

Fig. 5. The contractions are isometric and tetanic, lasting 5 seconds each. $A + B$ is a measure of the contraction in response to stimulation of all the fibers in the nerve. A and B record the contractions secured through stimulating the two components successively. The lever is calibrated and this is a type of the experiments summarized in table 1.

cord. Lindhard (4) has stated that the presence of two end-plates on one muscle-fiber is a condition frequently found in the frog.

We have, therefore, to explain the fact that we can obtain two successive fatigue records from a muscle in which the great majority of the fibers must have been at work through both series. We see no escape from the conclusion that what we fatigue is not the contractile mechanism itself but something interposed between this and the impulse arriving over the nerve. The temptation is strong to fix upon the end-plate as the seat of such fatigue. The definite anatomical structure appeals to one's desire for concreteness. But we are not warranted in naming any morphological feature; that which fatigues may be a portion of the chemical system within the fiber. Yet we must not lose sight of the fact that its fatigue must be local and partial since the closing of one approach leaves another unobstructed.

TABLE 1

The two components of the sciatic nerve are here called A and B. This is an arbitrary practice since we have not taken pains to identify them in an an anatomical sense. In the first column are recorded the tensions in grams developed when one of the two bundles is maximally stimulated; in the second the corresponding figures for the companion. In the third column are the tensions produced in each case by maximal stimulation of all the motor fibers at once

A	B	A + B
52	56	60
48	60	64
44	36	48
32	40	40
72	64	80
60	56	68
52	60	68
64	52	68
80	60	84
36	40	44
40	32	40
40	32	40
48	40	52
Average.....51.4	48.3	58.1
Per cent of total.....88.4	83.1	(100)

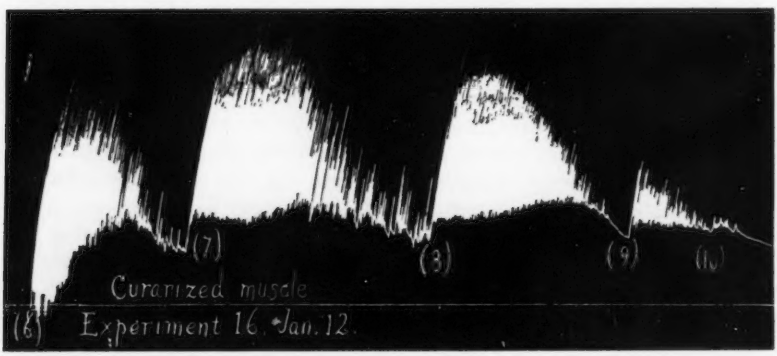

Fig. 6. Isotonic contractions. The muscle is stimulated directly and whenever its responses are much reduced the strength of the stimuli is increased. The numbers show the times of moving the secondary coil.

There is a certain resemblance between the situation indicated and the type of organization postulated for the central gray matter. The fatigue of a reflex arc is held to be synaptic and Sherrington (5) and Forbes (6) have long since shown that if stimulation is transferred to a fresh sensory path the action which has failed may be renewed. The final common path can be reached by an indefinite number of afferent approaches. It now appears that a muscle fiber is accessible at more than one place to the motor impulse. When the electric current is led directly through the tissue additional points must become available for the initiation of the excitatory process.

It is interesting, though premature, to speculate on the ways in which the plurisegmental equipment may be utilized in life. Is the innervation of muscle-fibers sustained by alternating activity in different segments? Or is it effected by one segment until fatigue has developed and then continued by another? Clearly, the arrangement is not adapted to reinforce brief contractions but rather to prolong the time during which work can go on. Suggestions multiply in regard to the relation of such an organization to training, second wind, the reëducation of patients with central lesions, and so forth.

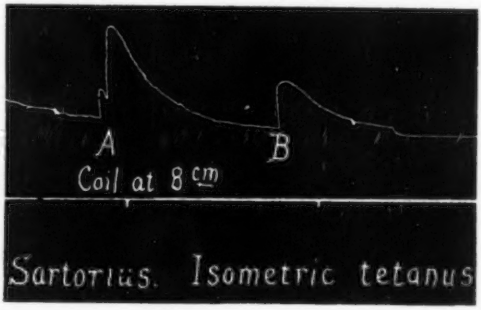

Fig. 7. A sartorius is stimulated to apparent exhaustion with electrodes placed near one end, *A*. A considerable rally is obtained when the stimulation is transferred to a distant part of the muscle, *B*.

There is abundant evidence to show that the muscle fatigue commonly registered in the laboratory is a *fatigue of excitation*. Many physiologists must have been impressed by the fact that increasing the strength of direct stimulation may repeatedly force a flagging muscle to contract nearly to its initial maximum. Records making this obvious have been published by Polimanti (7) and by Pereira (8). Our own figure 6 is an illustration of the same possibility. To a late stage in such a trial the power to make a full-sized contraction is latent in the tissue but it is increasingly difficult to command it.

We have some evidence that shifting the points of application of a stimulating current, applied directly to a muscle, may produce revivals of contraction even in cases where the entrance of fresh fibers into action appears improbable. Thus with the sartorius it is possible completely

to fatigue the muscle by stimulation brought to bear on the proximal half and later to excite a considerable contraction by passing the current through the distal part. Figure 7 is an example. At *A* a maximal isometric tetanus is recorded with the electrodes on one region of the muscle; at *B* there is seen the result of a shift to another locality. The obvious criticism of the attempt is that there may be fibers limited to the second section of the muscle which escape stimulation during the first part of the experiment. But this can hardly be true on any extensive scale for we can get as intense an isometric contraction from the fresh sartorius by stimulating it near one end as we can by using two pairs of electrodes at once and spacing them as in the two-stage procedure.

One type of experiment which we thought might confirm our general impression has signally failed to do so. This consists in fatiguing a muscle by a series of break-shocks and then reversing the current. It was anticipated that this would be equivalent to a relocating of the numerous physiological poles which mediate stimulation. The failure may be due to the vast number of these poles in the path of the current; there may be no areas sufficiently removed from them to function as fresh resources.

From the standpoint of economy it appears that supramaximal stimulation of a muscle must be unfavorable to its best performance. We have seen that more work can be gotten from the gastrocnemius when its two segmental nerves are stimulated successively than when both are brought into action simultaneously. By an extension of the principle to direct stimulation we are led to assume that the excitatory part of the system must be used up prematurely and to no purpose when the current employed is needlessly strong. In six experiments we compared the work done by the right and left gastrocnemii treated as follows. One muscle was subjected directly to 24 break-shocks a minute with the coil in a position to give a decidedly excessive current. The companion muscle was stimulated at the same rate with the coil so manipulated as to insure maximal responses but not to overstimulate. This is to say, in the second case the coil was moved toward the primary by short steps whenever the decline in the height of the contractions made it seem necessary. A work-adder was used to secure a basis of comparison. The muscle thus guarded against overstimulation always outlasted its fellow and exceeded it on the average by 40 per cent in total work performed.

CONCLUSIONS

1. In the frog the gastrocnemius muscle is innervated from two spinal segments. The tension developed by the muscle when either root is stimulated alone is nearly as great as when both are stimulated simultaneously. This indicates that a majority of muscle fibers receive nerve elements from two segments of the cord.

2. Stimulation of the muscle through one nerve root until contraction no longer occurs does not appreciably affect the performance of the muscle when stimulated through its other nerve component, i.e., a second fatigue curve comparable with the first may be obtained. The muscle may thus be made to do more work when its two nerve components are stimulated successively than when both are stimulated at the same time.

3. These facts lead to the conclusion that muscular fatigue as observed in the laboratory is not usually due to an exhaustion of the power of contraction, but to a failure of the process of excitation in consequence of some sharply localized change.

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BLOOD SPECIFIC GRAVITY: ITS SIGNIFICANCE AND A NEW METHOD FOR ITS DETERMINATION

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THE METHOD. Methods for the determination of the specific gravity of small quantities of blood have hitherto been based upon the principle of finding a mixture of suitable fluids whose specific gravity is such that a drop of blood will neither rise nor fall therein. Obvious advantages will attach to a method in which the whole procedure can be accomplished with the use of one drop of blood in a single mixture and in less than a minute's time.

The general principle of the following procedure involves timing the fall of a drop of blood of known size through a definite distance in a mixture non-miscible with blood. Such a mixture should have low viscosity and a specific gravity not over 0.030 lower than that of the blood to be tested. The pair of substances (one heavier and one lighter than the desired range of specific gravity) employed for this purpose should, furthermore, agree fairly well as to volatility so that accidental evaporation will not seriously disturb the balance. After trying a number of different combinations and after correspondence with Dr. H. T. Clarke of the Department of Synthetic Chemistry of the Eastman Kodak Company, we have been unable to find a more suitable combination than that of xylene (density 0.861–3, B. P. 136°–141°C.) and bromobenzene (density 1.495, B. P. 156°). Both of these substances are quite insoluble in water and give no visible evidence of interaction with a drop of blood exposed to a mixture of them for several minutes.

The apparatus needed includes a 100 cc. burette (of 15 mm. bore) and a Westphal specific gravity balance, the bob of which is suspended in a control test tube containing a mixture identical with that in the burette. The fluid in the control tube may, if desired, be kept in continuity with that in the burette. Owing to the high coefficient of expansion of suitable mixtures, their specific gravities vary decidedly with slight temperature changes. The Westphal balance serves, therefore, to control all such variations at the time of each observation. In spite of this, however, it is well to avoid sudden changes in temperature by

draft, sunlight, and even the heat radiating from the body. A little saturated solution of sodium sulphate in the bottom of the burette serves to catch the blood drops, preventing the formation of serum which otherwise might float up through the fluid. The burette is closed at the lower end by a rubber stopper, protected by a drop of mercury from the action of the fluid.

Great importance attaches to the size of the drop of blood and the manner in which it is introduced into the burette. As a blood pipette, we have used a piece of capillary tubing ground to a point and calibrated to deliver between marks 45 c. mm., with the usual mouth piece and rubber tubing. For easy delivery, such a pipette should not be constricted at the point. Frequent cleansing with ammonia, distilled water, alcohol and ether is recommended. Having secured a drop of freely-flowing blood, which must contain absolutely no trace of air, the specified quantity is delivered just beneath the surface of the xylene-bromobenzene mixture in the burette. *Should any air become included in the process of delivery, the results will be valueless.* Another source of error consists in small xylene-brombenzene inclusions in the drop of blood. Therefore care must be taken that no xylene-bromobenzene finds its way into the pipette.

The drop of blood is released by quickly and gently removing the pipette from the fluid. The fall of the blood drop over a stretch of 60 cm., beginning from 4 to 5 cm. below the top of the fluid, is determined with a stop watch. The speed of a falling drop will evidently vary in the same direction as its specific gravity.

Calibration. While it might seem desirable to translate the falling time into terms of specific gravity by the use of blood, it was found more feasible to establish this curve of relationship by using samples of saline of known specific gravity. It was then established that the values for blood could be superimposed upon this curve. Such a curve is found in figure 1. The abscissae indicate the number of seconds falling time while the ordinates express the difference between the specific gravity of the falling fluid and the surrounding mixture. Each dot in figure 1 indicates the results of averaging ten determinations of the falling time of a given sample of saline falling through xylene-bromobenzene having a specific gravity of about 1.030. Each circle represents a check made with oxalated dogs' blood.

The above mixture serves for all ordinary purposes but, to render the method more flexible, a xylene-bromobenzene mixture of about 1.010 specific gravity was used for further calibration. The fact that the points found can be superimposed upon the original curve (crosses) serves to show that considerable changes in the relative proportions of the two constitutents of the test mixture can be made without altering the values of the falling time. The last mentioned mixture would of course be suitable for testing anemic blood.

Illustrative protocols. To indicate the rapidity and accuracy of the method for determining blood specific gravity, table 1 is introduced, showing a series of eight observations in a resting splanchnotomized dog, checked by determinations of the blood solids from two different samples, all within nine minutes. This protocol serves further to illustrate the use of the calculation curve.

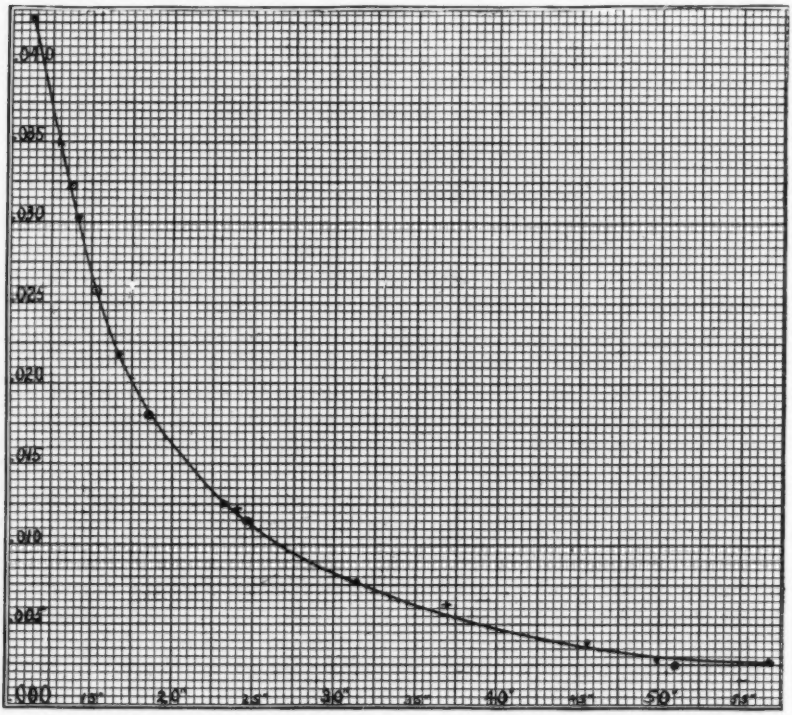

Fig. 1. Calculation curve for specific gravity of blood. Abscissae: fallig time in seconds; ordi nates: difference between specific gravity of falling drop andn xylene-bromobenzene. Details in text.

In table 2 are illustrated the effects of emotional excitement and epinephrin injection upon the specific gravity of the blood. The significance of these changes will be discussed in another paper.

THE RELATION OF BLOOD SOLIDS TO SPECIFIC GRAVITY. Were water the only factor concerned in qualitative blood changes, either total solids or specific gravity determinations would serve for their detection. Since, however, solutions of the two most important constituents of the blood,

TABLE 1

Blood specific gravity and total solids in resting splanchnotomized dog

Dog. 7. May 28, 1924.

TIME	SECONDS FOR BLOOD TO FALL 60 CM.	SPECIFIC GRAVITY XBB MIXTURE	CORRECTION FOR FALLING TIME	SPECIFIC GRAVITY OF BLOOD	BLOOD SOLIDS
a.m.					*per cent*
9:25	18.0	1.030	0.0187	1.0487	
9:26	18.0	1.0301	0.0187	1.0488	
9:26½					17.85
9:27½	18.2	1.0301	0.0184	1.0485	
9:28½	18.4	1.0301	0.0182	1.0483	
9:29½	18.4	1.0301	0.0182	1.0483	
9:32	18.0	1.0301	0.0187	1.0488	
9:32½					17.84
9:33½	18.2	1.0301	0.0184	1.0485	
9:34	18.2	1.0301	0.0184	1.0485	

TABLE 2

Effects of excitement and of epinephrin injection upon blood specific gravity in normal dog

Dog 11. May 26, 1924.

TIME	BLOOD	TIME	BLOOD
10:48½	1.0500	11:11	1.0504
10:50½	1.0510	11:17	Injection epinephrin*
10:51½	1.0517	11:19	1.0562
10:53	1.0507	11:19½	1.0559
10:58	1.0505	11:20	1.0550
10:59–11:01	Excitement	11:20½	1.0547
11:01½	1.0529	11:21	1.0542
11:02	1.0521	11:21½	1.0542
11:03	1.0502	11:22½	1.0523
11:04–11:05	Excitement	11:23	1.0528
11:06	1.0542	11:24	1.0533
11:07	1.0537	11:24½	1.0525
11:08	1.0525		
11:10	1.0504		

* 0.7 cc. of 1:10,000; 0.01 mgm. per kilo.

protein and salt, differ widely from each other in respect to their weight-volume relations, it becomes apparent that any changes in the proportion of these two substances must alter the relation between specific gravity and total solids.

Curves of this relationship for two differently constituted fluids appear in figure 2. Curve *A* has been compiled from data in the Smithsonian Physical Tables concerning the density of solutions of sodium chloride.

Curve *B* is constructed by connecting the point representing pure water with one which may be considered fairly representative of normal dog's blood. The three short lines shown at the upper end of this curve are based upon a series of estimations made under varying physiological conditions (detailed in fig. 3). These two curves, therefore, represent water changes in saline solution and blood, respectively. Obviously, a

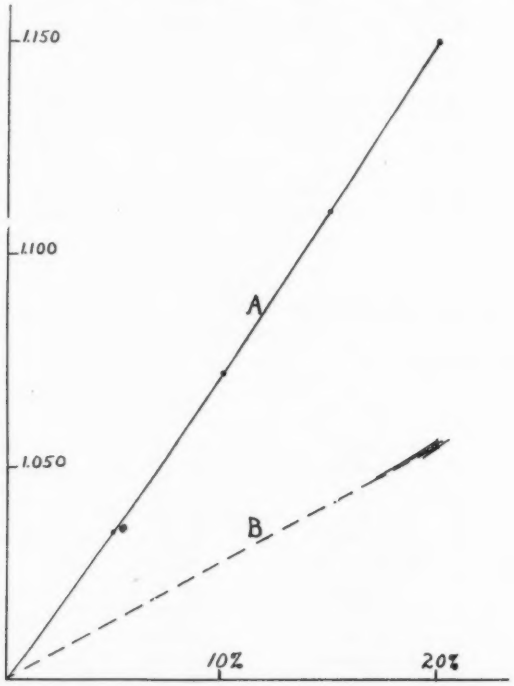

Fig. 2. Relation of solids to specific gravity in *A*, sodium chloride solution, *B*, blood (hypothetical). Abscissae: total solids percentage; ordinates: specific gravity. Lower left-hand corner represents pure water. Short lines condensed from figure 3.

curve for a solution of pure blood proteins would lie nearer to the abscissa than either of the curves *A* or *B*. It may be pointed out that the salt solution curve is not absolutely linear in direction and, undoubtedly, similar curvatures will appear when the blood and protein lines have been developed. An infinite series of similar curves could radiate from the pure water point between the salt and protein curves, each representing a definite $\frac{\text{protein}}{\text{salt}}$ ratio. For the purpose of establishing a general princi-

ple which concerns chiefly its protein and salt content, the other constituents of the blood may temporarily be ignored.

It therefore becomes possible to estimate the relative content of the blood in salt and protein by simultaneous determinations of blood solids and specific gravity.

Physiological variations. The relationship between blood specific gravity (ordinates) and solids (abscissae) under a variety of physiological conditions is illustrated in figure 3. Each of the lines, *C*, *D* and *E*, col-

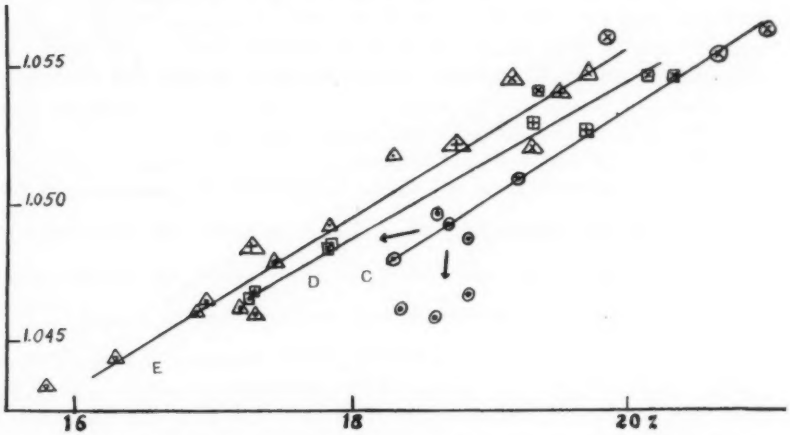

Fig. 3. Physiological variations in relation of blood solids to specific gravity. Line *C* (large circles): normal dog; line *D* (squares): same dog as *C* nine days after splanchnotomy; line *E* (triangles): another dog fifty days after splanchnotomy. Exposure to different environmental temperature conditions indicated as follows: *X*, 20°C. bath; +, 25°C. bath; ·, 29°C. bath; •, ordinary room conditions; ○, hot moist chamber, 35°C.

Upper arrow: decrease in $\frac{\text{protein}}{\text{salt}}$ ratio due to splanchnic anemia.

Lower arrow: increase in $\frac{\text{protein}}{\text{salt}}$ ratio due to hot environment, seen in normal dog only.

lates a series of determinations in a single experiment. The determinations relating to curve *C* ○, were made from a normal dog. Curve *D*, □, was similarly compiled from the same dog nine days after double splanchnotomy, while curve *E*, Δ, represents an experiment upon another dog, a still longer period (fifty days) after double splanchnotomy. In order to show the relationship between blood specific gravity and solids under widely varying conditions of blood concentration, the animals were in each case exposed to a series of different environmental temperature

conditions. The points indicated by X represent observations made with a dog in a bath up to the neck, at 20°C.; those indicated by + in a similar bath at 25°C.; those indicated by small dots, ·, in a bath at 29°C. The large dots, •, are all from observations made under ordinary room conditions, while the small circles, ○, indicate the results of placing an animal in a fairly moist environment of about 35°C. (wet-kata cooling power in the neighborhood of 8–10 millicalories per sq. cm. per second). In each experimental series is seen a trend from concentration to dilution as the environmental conditions became progressively warmer.

There were two reasons for using the splanchnotomized dogs in these experiments: 1, they show a progressive anemia which is not related to hemorrhage or to diet conditions; 2, because these dogs do not show large variations in blood concentration due to excitement. Detailed substantiation of these statements will appear later.

Although the trend of each experiment indicates clearly a mere displacement of water into or out of the blood without disturbance of the $\frac{\text{protein}}{\text{salt}}$ ratio, two distinct instances emerge in which this ratio appears markedly to alter. As indicated by the upper arrow, the anemic blood of the splanchnic dogs shows a marked decrease from the normal $\frac{\text{protein}}{\text{salt}}$ ratio. This shift is strikingly substantiated at whatever level of environmental temperatures one compares the observations. The dog which has been for the longest deprived of the splanchnic nerve connections shows both the greatest anemia and the lowest $\frac{\text{protein}}{\text{salt}}$ ratio.

The second change in the $\frac{\text{protein}}{\text{salt}}$ ratio is indicated by the lower of the two arrows, which represents the shift occurring on exposure of the normal dog to the hot environment. A decided increase of the $\frac{\text{protein}}{\text{salt}}$ ratio is indicated. It appears, from these few observations, that the mechanism controlling this shift in the solid constituents of the blood is eliminated by cutting the splanchnic nerves, for after this operation the hot room observations fall upon the same $\frac{\text{protein}}{\text{salt}}$ curve as those made at ordinary room temperatures, indicating simple dilution of the blood.

SUMMARY

1. A method is described for the determination of the specific gravity of the blood in which each observation requires but one drop of blood and less than one minute's time, including the calculation.

2. This method is based upon the falling time of a drop of blood of known size over a definite distance, through a mixture of xylene and bromobenzene which, for ordinary purposes, should have a specific gravity of approximately 1.030.

3. The relation of blood specific gravity to total solids is by no means a constant one. Evidence is presented indicating that simultaneous determinations of these two factors afford a method for estimating changes in the $\frac{\text{protein}}{\text{salt}}$ ratio.

4. Cutting both the splanchnic nerves renders dogs' blood anemic, increasing the water content and decreasing the $\frac{\text{protein}}{\text{salt}}$ ratio. In such dogs this ratio is not affected by marked changes in environmental conditions.

5. Normal dogs may possess a mechanism which increases the $\frac{\text{protein}}{\text{salt}}$ ratio in hot environments and is removed by cutting the splanchnic nerves.

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